VOLUME XIV JULY, 1931 NUMBER 4

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

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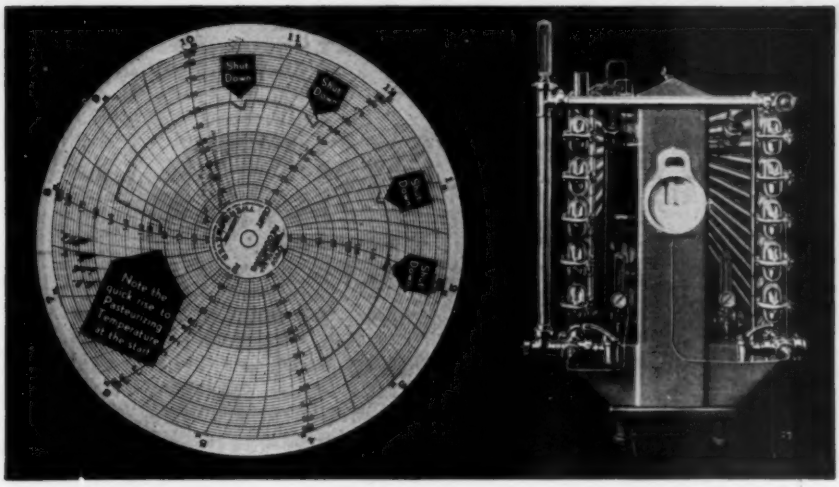

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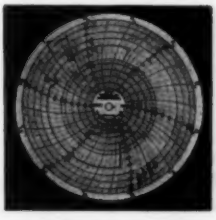

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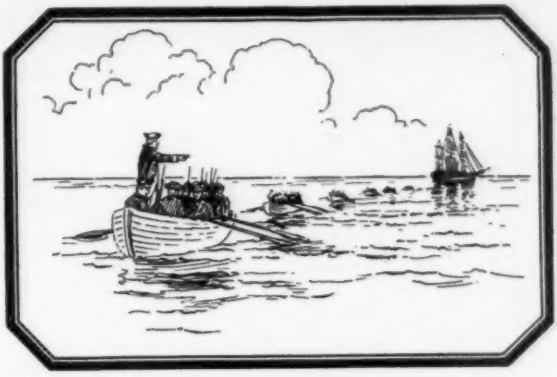

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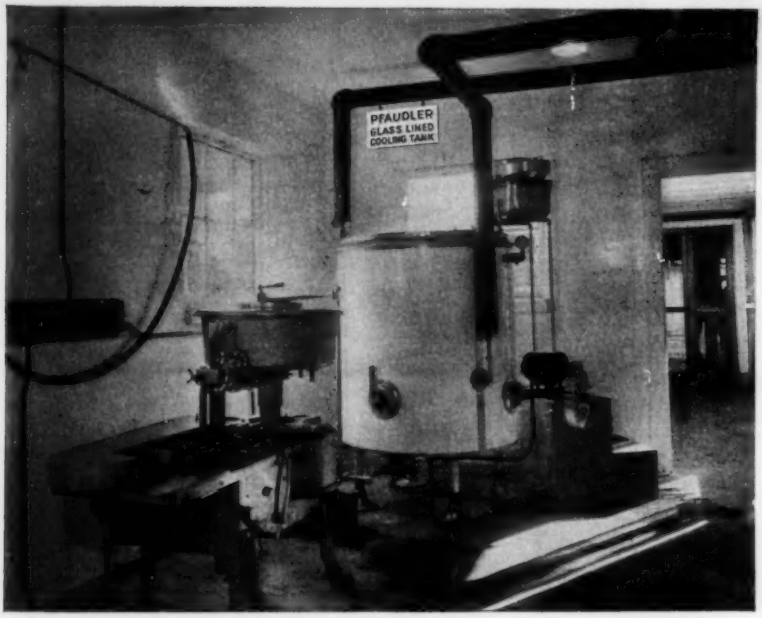

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DEFICIENCIES IN RATIONS DEVOID OF ROUGHAGE FOR CALVES*

I. THE EFFECT OF THE ADDITION OF COD LIVER OIL AND ALFALFA ASH

S. W. MEAD AND W. M. REGAN

Division of Animal Husbandry, University of California, Davis, California

That the failure to secure continued normal growth in dairy animals reared on rations devoid of roughage results from a deficiency of Vitamin A alone or in conjunction with an inadequate supply of certain minerals, and not from a lack of roughage or bulk *per se*, is the conclusion to be drawn from the first of a series of experiments now being conducted by the Division of Animal Husbandry of the University of California at Davis.

Attempts have been made by a number of investigators to rear calves on milk alone or on milk plus some concentrate mixture designed to furnish all of the known requisites for their growth and well-being. Most of these experiments have failed; none has been entirely successful. The calves have either died at an early age or developed various deficiency symptoms and failed to make normal growth.

As early as 1897, Davenport (1) concluded that satisfactory growth and development could not be secured when calves were fed rations containing no roughage. Twenty-five years later these findings were corroborated by McCandlish (2), who states: "The lack of bulk may arrest the development of the alimentary tract and prevent the proper digestion of the nutrients supplied by the milk." Results of investigations by Huffman (3) indicate that the beneficial element in roughage must be something other than bulk of crude fiber. He states: "Coarse feeds in the form of corn cobs, oat hulls, and shavings, when added to concentrate rations have failed to prevent the onset of convulsions. Ap-

* Received for publication November 11, 1930.

parently, hay furnishes some factor other than bulk which is essential in the rations of cattle."

Although Bechdel, Eckles, and Palmer (4) succeeded in raising dairy heifers to freshening time on a ration containing no hay, they used beet pulp to furnish roughage. At eight months of age, their calves developed typical symptoms of Vitamin A deficiency; but when cod liver oil was added to the ration, normal growth and development were secured. After the second week of lactation, further serious difficulties arose. The animals refused to eat and lost weight rapidly. All remedial measures except the feeding of hay proved ineffective. These workers conclude: "That the difficulty is due to a mere lack of bulk or roughage in the ration, rather than to a mineral or vitamin deficiency, we are not in position to say. It appears that the trouble may be the same as that reported by Reed and Huffman (8), McCandlish (9), and other investigators (10)."

In an attempt to grow calves without roughage a series of experiments was initiated, the first of which is described in this paper.

The animals used in this investigation were males dropped in the University herd. Calves 23E, 26E, 28E, 30E, and 31E were pure-bred Jerseys; calves 27E, 29E, and 32E were pure-bred Holsteins. They were placed on experiment at two days of age and with one exception were muzzled at this time. No. 23E did not have a muzzle until he was a month old.

All calves received whole milk from birth to six months of age. They received in addition a concentrate mixture, fed *ad libitum*, which consisted of the following feeds: 400 pounds of rolled barley, 300 pounds of rolled oats, 300 pounds of wheat bran, 100 pounds of linseed meal, and 8 pounds of salt. In place of roughage, calves 26E, 27E, 28E, 29E, 30E, and 31E received an amount of alfalfa ash equal to that supplied by a normal alfalfa hay ration. Data obtained from a number of feeding trials where calves received a normal ration were used in calculating the amount of alfalfa ash to feed.

The ash was obtained by burning alfalfa hay in iron drums with wire screen covers, which prevented the escape of a large

part of the finer particles. Foreign material was then removed by sifting.

Until the calves were six months old, the ash was reburned in an electric muffle. The daily allotment, as calculated for each calf, was placed in individual containers. For the first six months it was administered orally by hand; subsequently it was mixed with the concentrates at each feeding.

Calf 23E received milk to six months, as did the rest of the calves, but only concentrates thereafter; while 32E was allowed, in addition to the concentrates, copper sulphate and ferric oxide in amounts sufficient to prevent nutritional anemia, as calculated from the requirements of small animals (5).

An accurate account was kept of the amount of feed eaten by each calf. In order to determine the rate of growth, body weight, heights at withers, and heart girth measurements were taken at intervals.

The responses noted in the calves are presented in table 1; a record of growth, as indicated by body weight, is given in table 2. Figure 2 shows graphically the growth of each calf as compared with Eckles' normal (6). As will be noted, the calves grew normally until they were between seven and nine months of age, when all showed signs of physical deterioration, indicated by impaired vision, marked loss of appetite followed by a decline in body weight, stiffness of the joints, and, in some cases, loss of muscular control. Certain calves were eventually unable to stand, and all scoured profusely at times. When these symptoms, which were apparently those of a Vitamin A deficiency, became acute, "Patches" tested cod liver oil was administered in doses of 25 cc. daily. As the animals grew older it was deemed advisable to increase the dose to 75 cc. The clinical records of the individual calves follow:

Calf 23E. This calf was always below normal in body weight after 240 days of age. His eyes began to water profusely at 229 days; a white spot appeared upon each cornea, and complete loss of sight ensued. This condition was accompanied by rapid respiration and a profuse nasal discharge. The appetite completely failed, and the animal became very much emaciated; its joints became quite stiff. Beginning at 289

days of age, cod liver oil was administered. Within one week there was a marked improvement in appetite, the eyes stopped watering, and the sight became apparently normal. At 523 days of age the daily administration of cod liver oil was increased to 75 cc.

Calf 26E. The clinical picture was much the same as that of 23E, except that about three weeks were required for eyesight to become normal after the first dose of cod liver oil was given at 256 days. That

FIG. 1. CALF 29E

This condition, which developed in most of the calves at seven to nine months of age, was relieved immediately by the administration of cod liver oil.

the breeding powers of this bull were not affected at 394 days of age is proved by the fact that a heifer bred to him became pregnant. At 490 days of age the cod liver oil dosage was increased to 75 cc. daily.

Calf 27E. This was the only animal to die during the experiment. Post mortem examination showed the immediate cause of death to be a severe case of pleurisy. From 60 days of age, this calf made better than normal growth and showed no loss of appetite until ten days before death. He developed eye trouble at 232 days of age, the right eye

suddenly turning white. His joints became stiff at 245 days of age. He was given cod liver oil at 257 days of age but died the next day.

Calf 28E. At 252 days of age, this animal began to exhibit a marked decline in appetite, and at 255 days, a loss of muscular control and impaired vision. No white spots appeared in the eyes, but at 291 days of age, he was in such poor physical condition that he was given cod liver oil. A gain in appetite was evident in 2 days; within a week his physical condition was greatly improved, and his eyesight was restored. The cod liver oil dosage was increased to 75 cc. daily at 450 days of age.

Calf 29E. Though there was no noticeable decline in appetite until 254 days of age, loss of muscular control and impaired vision were evident by the 248th day, and convulsions occurred on the 270th and 271st days. At this time he was totally blind; the left eye was matterated and white, badly ulcerated, and blood-shot. As shown in figure 1, the eyes were closed most of the time. Cod liver oil was administered on the 272nd day. Within 7 days, sight was restored to the right eye, which appeared normal. In another week there was considerable improvement in appetite and general vitality. By the time this calf was 292 days of age, vision had been completely restored, though a small white spot remained in the left eye. The rate of growth was also greatly accelerated. When 314 days old, however, he again began to evidence decline in appetite, to fall off in body weight, and to exhibit eye trouble. His daily dosage of cod liver oil was increased to 75 cc. He refused all feed for 15 days. In spite of the fact that an effort was made to stimulate his appetite by giving 6 to 8 pounds of whole milk daily, he lost 141 pounds in 30 days. During the next 10 days, he regained 54 pounds, and at 360 days of age he was eating well and rapidly gaining in weight.

Calf 30E. This animal showed decline in appetite at 230 days of age, though there was no noticeable loss in muscular control or stiffness of the joints. At 263 days, he had entirely lost his vision; and his watering and matterated eyes were closed most of the time. Cod liver oil in the usual amount was administered at 267 days. Within 2 days he exhibited a decided improvement in general health; the left eye was open and appeared clear and bright, though the right eye was still affected. By the time the calf was 276 days old, sight had been restored to both eyes; and he was eating well and gaining rapidly in weight. At 426 days of age the daily amount of cod liver oil was increased to 75 cc.

Calf 31E. Decline in appetite was evident at 226 days of age, and

muscular control was gone by the 258th day. When 276 days old, he was totally blind. Two days later, cod liver oil was administered. Within a week his vitality had improved, and he had regained control of his movements; but he remained blind. At 414 days of age the cod liver oil dosage was increased to 75 cc. daily.

Calf 32E. This animal received copper and iron in place of alfalfa ash. In attempting to enter his stall at 216 days of age, he became jammed in the gate, injuring his hip. Though for a few days he was only slightly lame, the condition became accentuated, and after a month he remained in a prone position most of the time. His appetite began to fail at 222 days, and his eyes watered, though his vision at no time seemed to be badly impaired. Greatly emaciated, he appeared to be on the verge of death at 245 days of age, when cod liver oil was administered in the usual amounts. Improvement was slow; but by the 268th day his appetite had improved, and by the 290th, he was able to walk, though his left hip showed evidences of being broken. He gained in weight rapidly to 360 days of age, when the lameness was again accentuated and loss in weight occurred. Later he regained some of the loss but was still below normal in body weight. At 402 days of age his cod liver oil dosage was increased to 75 cc. daily. His rate of growth increased so that at 500 days of age he was slightly above the normal in body weight.

It is appreciated that the number of animals used in this experiment is small. Complete individual records, however, were taken; and, as shown in the data presented, almost identical results were obtained. All calves developed the deficiency symptoms at approximately the same age and in a strikingly regular sequence. When the remedial measure was taken, the steps in recovery were made in all cases in the same order and at uniform intervals.

As far as the authors have been able to ascertain from a search of the literature, this is the first time that a group of calves has been reared to the age of 19 months on a ration devoid of roughage, and normal growth secured. While other investigators have indicated that bulk is unnecessary, no one has proved it so by successfully growing the calves without it. Although others have used with good results a ration containing no hay, they supplied roughage in the form of beet pulp. The experiment reported here

has demonstrated that calves can be grown to at least 19 months of age on a ration devoid of roughage. As shown by figure 2, five calves, except for short periods when they were suffering from Vitamin A deficiency, were above normal weight.

That the concentrates were inadequate in Vitamin A is evident from the fact that all calves developed marked symptoms of this deficiency, which disappeared upon the administration of cod

TABLE 1

Deficiency symptoms and responses to cod liver oil administration observed at ages indicated

CALF NUMBER	STIFFNESS AND LOSS OF MUSCULAR CONTROL	MARKED LOSS OF APPETITE	SIGHT AFFECTED	COD LIVER OIL ADMINISTERED	FIRST RESPONSE	SIGHT NORMAL
	age in days	age in days	age in days	age in days	age in days	age in days
23E		240	229	289	296	296
26E	Stiffness, 210	235	225	256	263	296
27E	Stiffness, 245	248	232	257	Dead at 258	
28E	Loss of muscular control, 255	252	255	291	293	297
29E	Loss of muscular control, 248	254	248	272	279	292
30E		230	263	267	269	276
31E	Loss of muscular control, 258	226	262	278	Increased vitality, 285 Complete muscular control, 313	
32E	Injured hip, 216	222	222	245	Appetite improved, 268 Able to walk, 290	

liver oil. But that Vitamin A is not the only essential which the concentrates failed to supply in adequate amounts is indicated by the fact that calves 23E and 32E, which received no ash, were considerably underweight during most of the experiment.

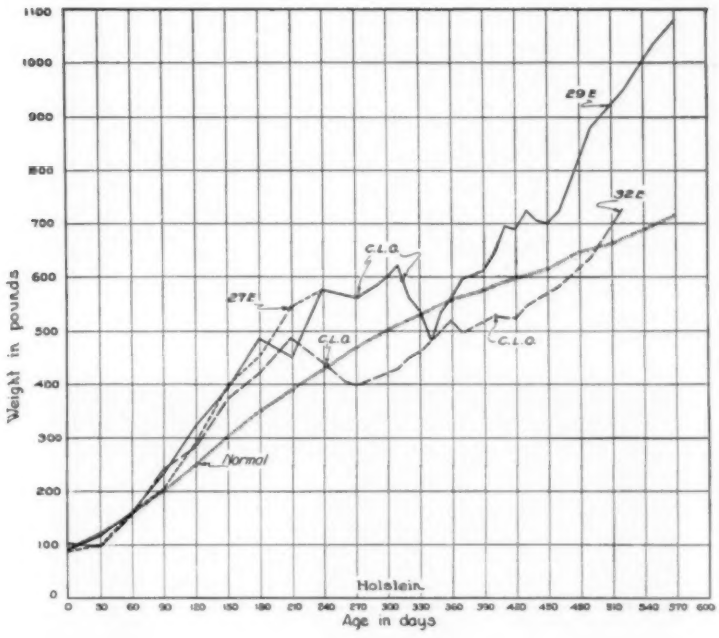
CONCLUSION

Calves can be reared to 19 months of age and normal growth secured on a ration containing no roughage, providing cod liver oil and alfalfa ash are supplied in sufficient amounts.

TABLE 2

Body weights

AGE	CALF 23E	CALF 26E	CALF 27E	CALF 28E	CALF 29E	CALF 30E	CALF 31E	CALF 32E
days	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Birth	46	54	87	49	90	57	55	102
30		52	98	80	115	62	59	96
60	73	67	162	105	160	114	112	157
90	105	123	203	165	233	182	182	240
120	172	160	290	250	321	220	200	282
150	212	235	398	285	390	290	245	372
180	295	330	450	341	484	355	270	420
210	350	370	544	400	450	400	335	486
240	337	409	575	393	575	397	332	440
260						353	350	403
270	324	380		360	561	381	355	397
280						390	367	
290				343	584	408	383	
300	311	438		374	599	421	393	
310				384	620	429	425	428
320				397	560	461	442	449
330	298	522		408	535	463	464	460
340				422	479	485	467	480
350				457	533	482	483	500
360	360	518		463	561	493	508	518
370	370	540		469	597	499	517	495
380	356	559		491	601	509	531	504
390	377	584		510	610	513	537	516
400	373	583		518	643	533	559	527
410	392	614		525	693	518	544	523
420	382	609		550	688	517	533	523
430	392	643		566	722	504	529	546
440	383	637		551	702			
450	380	640		541	698			
460	409	639		541	721	530	575	580
470	407	665						
480	409	661						
490	436	683		610	880	584	622	634
500	448	686						
510	457	667						
520	446	695		610	948	660	665	725
530	446						673	
540	451					703		
550		675		635	1035			
570	485			662	1080			
580		707						
600	435							
610		775						
620	500	774						



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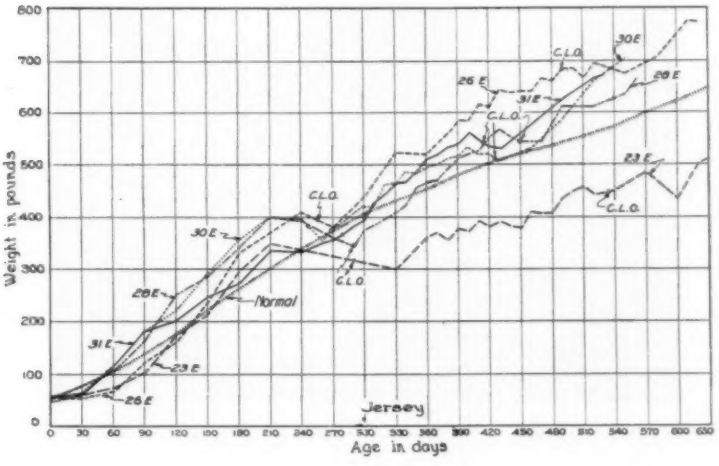


2B

FIG. 2. INDIVIDUAL RECORDS OF GROWTH AS MEASURED BY BODY WEIGHT COMPARED WITH ECKLES' NORMAL

Calf 23E received milk and concentrates. 32E received, in addition, copper sulphate and ferric oxide; all others, milk, concentrates, and alfalfa ash.

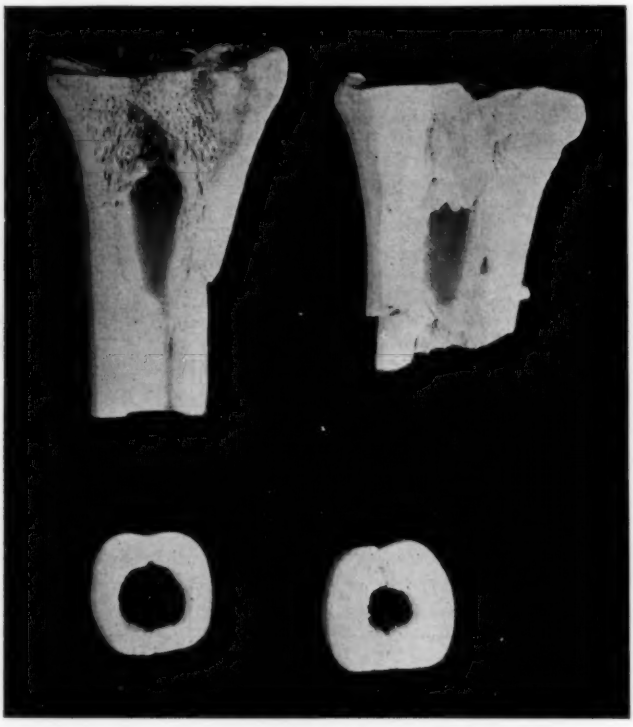
TABLE 3

Analyses of canon bones of representative animals

ANIMAL NUMBER	WHEN SLAUGHTERED		DRY FAT-FREE BONE		MINERAL SUPPLEMENT
	Age	Body weight	Weight	Ash	
	days	*pounds*	*grams*	*per cent*	
23E (J)*	758	665	203	59.8	None
32E (H)†	647	925	284	53.8	None
26E (J)	725	904	264	66.5	Alfalfa ash
29E (H)	677	1,335	473	62.4	Alfalfa ash

* J—Jersey.

† H—Holstein.



No. 23 E No. 26 E

FIG. 3. THE DEPOSITION OF MINERALS IN THE CANON BONES OF ANIMALS ON A ROUGHAGE-FREE RATION

No. 23E received no mineral supplement, while No. 26E received alfalfa ash.

ADDENDA

Because of limited barn facilities and the difficulties encountered in the management of two-year old bulls, the experimental animals reported upon above were slaughtered after the original manuscript had been submitted for publication. Data taken at the time of slaughter was, in the opinion of the authors, of sufficient importance to merit publication as addenda.

The following conditions were noted: A low deposition of minerals in the bones of 23E and 32E was indicated by the fact that their spinal columns could be readily cut with a knife, and that the canon bone of one was crushed while hoisting the carcas to the track. This was verified by the analyses of the left hind canon bones of representative animals as shown in table 3 and by figure 3. It is interesting to note also that the animals making the greater gain in each group showed the lower percentage of ash in the dry fat-free bone. The caul fat of all the animals appeared pure white. While they lacked the usual fill, the digestive tracts were otherwise normal. The dressing percentages were unusually high, averaging 57.4.

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LENGTH OF CALVING INTERVAL AND AVERAGE MILK YIELD*

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The term "calving interval" refers to the period from one calving to the next calving. In dairy practice the most common calving interval is in the neighborhood of one year. Two somewhat different considerations may be responsible for this practice. There may be an economic advantage in having the flush of milk production, associated with calving, occur at some particular time of the year. There may be a biological advantage in having reproduction occur with a certain frequency. This paper is concerned with the latter consideration.

It is well known that delayed breeding and a consequent long calving interval is conducive to a large 365-day record, and the system of advanced registry testing has consequently favored such a practice. On the other hand, it is generally held that shorter calving intervals favor a large yield for the lifetime of the cow.

That frequent calving should give maximum lifetime yield seems in harmony with the known facts of the physiology of reproduction and lactation. The natural need of milk develops with birth of the young. The need increases with growth of the young so long as milk is the only food. As other food is taken the need for milk decreases and finally disappears. In milch animals, where we have adequate information as to the amount of milk produced day by day, it is found that the rate of milk secretion parallels roughly the rising and declining needs of the young. That is, the rate of milk secretion is most rapid in the cow shortly after calving and slows up quite markedly and continuously thereafter. It would seem that frequent calving by giving more high points in the lifetime lactation curve, should result in a greater total yield, as compared with less frequent calving.

Another way of putting the matter is to relate the length of the

* Received for publication November 19, 1930.

calving interval to the average yield of milk per day over the calving interval. This relation may be derived in relative terms from the known characteristics of the lactation curve. The indication is that the highest lifetime yield should be obtained from calving intervals of less than a year rather than from calving intervals of more than a year (1).

Matson (2) has recently pointed out an aspect of the problem which had not been considered before, namely, the effect of the calving interval on the *following* lactation. He states that while a short calving interval may go with high average yield for the current lactation, "it is paid for in the next lactation."

In view of the large expenditure of energy involved in the heavy milk secretion of improved dairy cows the point seems to be well taken. In the present paper the writers have thought, therefore, to utilize certain published data by way of further test of Matson's point.

MATERIAL AND TREATMENT

Langmack (3) has published the lifetime records of 425 cows, including for each cow and each calving interval, the length of the calving interval in days, the total milk yield for the lactation in kilograms, and the fat percentage of the entire milk for the lactation. The material seems to be suitable to cast light on the effect of the length of calving interval with respect to both the current and the *following* lactations. Furthermore, by the method of partial correlation the data may be treated to show the effect of calving interval on current yield with preceding calving interval constant; and on the following yield with the accompanying calving interval constant.

A few symbols will aid in presentation:

I = calving interval in days.
I_1 = first I; I_2 = second I; etc.
Y = average yield per day over the current calving interval, pounds of 4 per cent milk.
Y_1 = Y for I_1; Y_2 = Y for I_2; etc.
f = lactation fat percentage.
f_1 = f for I_1; f_2 = f for I_2; etc.

We may then calculate $r_{I_1Y_1}$, $r_{I_1Y_2}$, $r_{I_1Y_3 \cdot I_1}$, $r_{I_1Y_3 \cdot I_2}$, and so on through the succeeding lactations. Finally, we may calculate $r_{I_nY_n}$ and $r_{I_nY_{n+1}}$, where n takes successively any or all values within the limits of the data. This sort of treatment should bring out the relations in question so far as these particular data go.

After some working with the problem it appeared desirable to select certain of the records to carry through. Most of the records (352) are for cows of the Red Danish breed; the other records are for cows of the Ayrshire, Holstein, and Jersey breeds. It is desirable to work within the same breed. For each cow the records give ten or more lactations, but the tenth lactation is often incomplete. Some of the records do not start with the first calf. Body growth in general and mammary growth in particular, are factors which are most active during the first calving intervals. Finally, there is a good deal of abortion. It is plain that abortion will tend to raise the yield per day for the interval which it terminates, and decrease the yield per day for the interval which it begins. It is especially desirable to stay clear of the effect of abortion.

There are 186 cows of the Red Danish breed which have records starting with the cow's first calf and covering the first 9 calving intervals, all ten calvings being normal, that is, no abortion. These records constitute a unique body of data adapted to solution of the present problem, and were selected for detailed study, with the results presented in this paper. The selection reduces the number of records, which is not desirable. On the other hand the selection should give a very comparable succession of lactations, with many variables automatically eliminated, and this advantage is regarded as more than offsetting the sacrifice of numbers.

It seems to be a sound principle to measure lactation by energy yield and fat percentage, the one as a measure of the amount of work done, the other as an index of the kind of work done (4). The fat percentage is given directly in the records. Energy yield is computed in terms of 4 per cent milk by the formula, $FCM = M(0.882 + 0.331f)$ in which FCM is 4 per cent milk in pounds, M is milk in kilograms, and f is fat percentage. This computation

together with the division for yield per day has been carried out by use of a 20-inch slide rule with an added fat percentage scale to fit the job. The conversion to pounds is made (with apologies) for the convenience of our readers who are mostly accustomed to thinking in terms of the English System.

RESULTS

The 186 cows under study were distributed among 13 different herds. In table 1 these herds are arranged in order of increasing

TABLE 1

Herd average calving intervals and yields for the first nine calving intervals

HERD NUMBER	NUMBER OF COWS	AVERAGE CALVING INTERVAL	AVERAGE 4 PER CENT MILK PER DAY	AVERAGE WEIGHT
		days	*pounds*	*pounds*
XIII	9	370	19.0	1,121
VI	4	375	14.4	981
IV	7	382	19.6	1,118
VII	2	382	13.9	
V	8	384	20.3	
XI	10	384	16.7	1,044
X	5	387	16.8	1,071
IX	11	391	16.5	1,079
II	34	403	16.7	1,017
VIII	12	404	18.5	1,185
III	22	410	17.8	973
I	43	411	16.7	1,046
XII	19	418	19.6	1,100
Average		401	17.5	1,061

calving interval to show the average yield per day over the first nine calving intervals, and the average weight per cow. The weights are given for only 155 of the 186 cows. This tabulation shows a range of 48 days in the herd average calving interval with a mean for all cows of 401 days.

The yield of 4 per cent milk shows considerable variation, but bears no particular relation to the length of calving interval. The average of all cows is 17.5 pounds of 4 per cent milk per day over the first nine calving intervals, which cover on the average

TABLE 2

Relation of length of calving interval to average yield of milk per day

The left-hand column shows the mid-points of the calving-interval groups by 20-day classes. The columns to the right show for the first 9 calving intervals of the 186 Red Danish cows, items as follows: n, the number of cows; Y_1, the average yield over the first calving interval in pounds of 4 per cent milk per day; Y_2, the same over the second calving interval; I_2, the mean length in days of the second calving interval; and so on to Y_9 and I_9. For example, dealing with the fourth calving interval there were 41 cows (n) with a calving interval of 350 days (column 1, line 5) that is, 340 to 359 days; the average yield of the 41 cows was 18.0 pounds of 4 per cent milk per day during the current calving interval (Y_4); and 18.2 pounds during the following calving interval (Y_5); the mean length of which was 402 days (I_5).

CLASSES	FIRST CALVING INTERVAL				SECOND CALVING INTERVAL				THIRD CALVING INTERVAL				FOURTH CALVING INTERVAL				FIFTH CALVING INTERVAL				SIXTH CALVING INTERVAL				SEVENTH CALVING INTERVAL				EIGHTH CALVING INTERVAL				NINTH CALVING INTERVAL	
	n	Y_1	Y_2	I_2	n	Y_2	Y_3	I_3	n	Y_3	Y_4	I_4	n	Y_4	Y_5	I_5	n	Y_5	Y_6	I_6	n	Y_6	Y_7	I_7	n	Y_7	Y_8	I_8	n	Y_8	Y_9	I_9	n	Y_9
270									1	21.9	16.5	385																					
290	1	11.9	14.0	355					1	19.1	19.5	425									1	23.5	17.5	385									
310	8	12.5	13.0	364	5	16.4	17.6	389	9	18.0	18.3	364	11	19.8	18.1	383	5	20.5	18.9	351	6	18.0	13.7	433	7	20.4	18.9	392	2	17.0	15.5	460	2	26.0
330	25	14.5	14.9	383	28	15.3	17.3	378	24	16.3	16.1	393	33	18.7	18.8	378	24	19.5	18.4	416	29	18.9	19.1	374	22	20.7	19.4	394	17	20.2	20.1	453	19	18.2
350	31	13.3	14.8	373	41	14.1	15.6	387	43	17.3	17.6	395	41	18.0	18.2	402	54	18.6	18.6	401	29	19.5	19.2	379	44	20.3	19.5	406	35	19.1	17.3	387	36	18.9
370	27	11.9	13.8	409	34	15.0	17.0	394	34	18.3	18.8	384	31	17.7	18.4	397	33	19.5	19.4	390	39	20.1	19.9	405	32	20.4	18.8	388	33	19.1	17.7	416	36	19.5
390	14	12.6	15.4	374	19	15.4	18.0	361	18	16.4	17.2	370	20	18.0	18.8	408	23	18.0	18.9	385	22	19.0	19.2	409	24	19.4	18.0	440	22	18.9	18.5	424	21	18.9
410	16	13.4	15.8	421	9	15.5	16.4	374	19	17.1	18.5	380	12	19.6	20.6	390	15	19.3	20.2	379	14	19.2	21.5	376	7	18.4	17.8	415	19	18.8	19.1	396	9	17.6
430	13	11.6	14.4	443	14	15.7	17.6	412	10	16.4	19.9	384	11	16.5	16.8	416	4	18.3	19.3	438	13	20.9	19.0	437	14	18.7	18.1	447	10	19.0	19.4	395	8	19.3
450	10	13.0	15.1	397	8	14.5	16.6	396	5	16.6	16.9	381	6	16.3	18.3	352	4	20.3	18.5	460	6	18.2	19.8	353	3	20.2	19.8	448	12	20.0	19.8	435	12	18.9
470	8	14.3	16.8	439	2	15.9	20.0	370	3	16.3	15.2	362	1	19.5	16.5	515	3	18.5	17.8	355	8	17.4	18.9	410	11	18.0	18.6	449	7	18.9	17.6	441	9	17.5
490	5	10.9	14.7	431	3	17.5	20.0	342	3	19.1	21.5	368	5	18.3	21.5	353	0				3	15.8	19.2	378	2	18.0	20.0	420	3	17.2	16.8	435	5	16.7
510	6	9.3	13.2	392	4	17.7	19.4	360	8	15.1	21.9	413	1	19.5	25.5	315	3	15.5	21.5	412	2	21.5	22.5	375	6	17.8	19.8	465	5	18.9	18.5	447	7	17.8
530	1	12.4	18.0	335	4	17.0	21.1	390	2	16.4	19.0	425	0				4	20.5	23.8	378	3	19.2	20.5	405	1	15.5	19.5	365	4	16.0	19.5	425	4	16.8
550	3	9.0	14.7	388	2	14.3	16.9	340	1	11.9	18.5	395	4	20.0	20.5	348	3	17.2	18.5	362	2	22.0	30.0	325	2	23.0	20.0	430	4	19.3	23.8	383	2	17.5
570	3	11.5	17.4	385	3	12.3	18.2	468	0				2	16.0	20.5	415	3	20.2	24.2	385	3	19.8	23.8	418	2	19.0	20.5	430	1	14.5	14.5	725	2	13.5

590	2	6.8	12.8	400	1	13.3	19.3	415	0				2	14.5	19.5	385	0				0				1	18.5	21.5	545	3	15.8	17.8	428	2	15.5
610	3	8.6	17.4	371	1	11.4	14.2	345	0				1	21.5	22.5	415	3	15.8	20.5	378	2	16.5	19.5	410	1	8.5	14.5	325	2	16.0	20.0	370	0	
630	3	11.6	15.7	398	4	13.5	15.2	583	1	18.2	19.5	385	2	18.5	22.0	545	0				1	20.5	16.5	465	0				0				3	19.8
650	2	10.4	18.7	485	2	13.7	17.8	365	1	15.9	19.5	555	0				0				2	15.5	17.0	590	1	13.5	15.5	425	0				2	15.5
670	1	9.0	12.0	375	2	15.5	19.8	360	1	19.5	23.5	385	1	16.5	20.5	475	0				0				1	14.5	23.5	365	1	16.5	22.5	365	1	20.5
690	2	10.2	18.7	405					0				0				2	17.0	22.0	355	0				2	14.0	15.5	365	0				2	20.5
710	0								1	16.5	20.5	405	0				0				0				0				4	16.3	20.8	495	1	6.5
730	1	15.2	18.5	545					0				0				1	11.5	19.5	425	0				0				0				2	16.5
750	0								0				1	16.5	22.5	355	1	17.5	27.5	385	0				1	13.5	16.5	665	0				1	22.5
770	0								0				1	12.5	28.5	375	0				0				1	15.5	20.5	385	0					
790	1	13.9	28.4	335					0								0				0				0				0					
810									1	9.4	18.5	625					0				0				0				0					
830																	1	8.5	17.5	425	0				1	18.5	19.5	395	0					
850																					0								1	21.5	17.5	325		
870																					0								0					
890																					1	16.5	21.5	345					0					
910																													0					
930																													1	19.5	24.5	385		

the first ten years of milking life. The average annual yield is therefore 6388 pounds of 4 per cent milk.

It is noticeable that the longer calving intervals occur in the larger herds, but the significance of this is not apparent. So far as can be seen from the herd averages of table 1 it should be permissible to throw the several herds together for the purpose of more detailed analysis.

The 186 records were sorted into classes on the first calving interval. For each of these classes were then computed: (n) the number of records in each class; (Y_1) the average yield per cow

TABLE 3

Calving interval, yield and fat percentage correlations

(For meaning of symbols see text, page 295)

n	COEFFICIENTS OF CORRELATION*							
	I_nY_n	I_nY_{n+1}	I_nI_{n+1}	Y_nY_{n+1}	Y_nI_{n+1}	I_nf_n	I_nf_{n+1}	f_nf_{n+1}
1	−0.286	+0.344	+0.124	+0.451	+0.031	+0.056	−0.065	+0.733
2	−0.012	+0.121	+0.167	+0.558	+0.078	−0.068	−0.113	+0.774
3	−0.138	+0.211	+0.173	+0.583	+0.007	+0.089	+0.016	+0.811
4	−0.133	+0.195	+0.049	+0.618	−0.015	+0.020	+0.034	+0.794
5	−0.208	+0.230	−0.045	+0.485	+0.024	+0.029	+0.100	+0.761
6	−0.097	+0.033	+0.073	+0.482	−0.087	+0.130	+0.077	+0.746
7	−0.312	−0.049	+0.132	+0.464	+0.076	−0.137	−0.098	+0.791
8	−0.156	+0.166	+0.051	+0.336	+0.019	+0.123	+0.127	+0.846
9	−0.169					+0.034		

* The probable errors range from 0.014 to 0.049.

during the current calving interval, in pounds of 4 per cent milk per day; (Y_2) the average yield per cow during the following calving interval, likewise in pounds of 4 per cent milk per day; (I_2) the average length of the following calving interval in days. The records were then sorted on the second calving interval and a similar procedure followed; and so on to the ninth calving interval. The results are given in table 2.

It is apparent from table 2 that as the calving interval increases the average yield per day tends to decrease during the current interval, and tends to increase during the following interval. But the relations are very irregular.

The various correlations have been worked out and are given in table 3. From this table we see that the correlation between calving interval and current yield per day is negative in each case; and, with one exception, it is positive when dealing with the following yield. In magnitude the coefficients are none very high and several are very low. There is a positive correlation between the length of one calving interval and that of the next, except in one case, but again the relation is not very pronounced. Such correlation as exists between the length of successive calving intervals might well be accounted for by the factor of herd practice rather than by any inherent tendency in the individual cow to a longer or shorter period in which conception may recur. The correlations, Y_nI_{n+1}, indicate that the yield during one lactation has no effect on the time of conception during the next lactation. Indirectly they may be taken to indicate also that rate of yield has no effect on the recurrence of conception at the time; and that there is no tendency to allow a longer calving interval to high yielding cows as compared with low yielding cows.

At this point we may interpose the results of correlating the average length of the first nine calving intervals with the average yield per day over the entire nine intervals. The coefficient, $r_{I_{1-9}Y_{1-9}}$, is 0.053 $\pm$0.049 for the 186 cows and the regression is linear. Considered on this basis calving interval has no effect on yield; and, again, high yielding cows are not bred to freshen less frequently than low yielding cows.

Turning to the correlation between the yields of successive lactations it is seen that between the first and second intervals, r = 0.451; the coefficient increases regularly to the fourth and fifth intervals where r = 0.618; it then decreases regularly to the eighth and ninth intervals, where r = 0.336. We have here a "growth and senescence" curve with respect to stability of milk secretion. In this particular the prime of life is reached at an earlier age than occurs with respect to the yield of milk. In the present set of records the maximum average yield per day is found during the seventh calving interval.

On account of the great irregularities shown in table 2 it has

seemed advisable to combine the several calving intervals to obtain a sort of average relation which may be more regular. In the relation expressed by $r_{I_n Y_{n+1}}$ age influences may disturb the result as a measure of the influence of calving interval. For example, of two cows each dropping her first calf at two years,

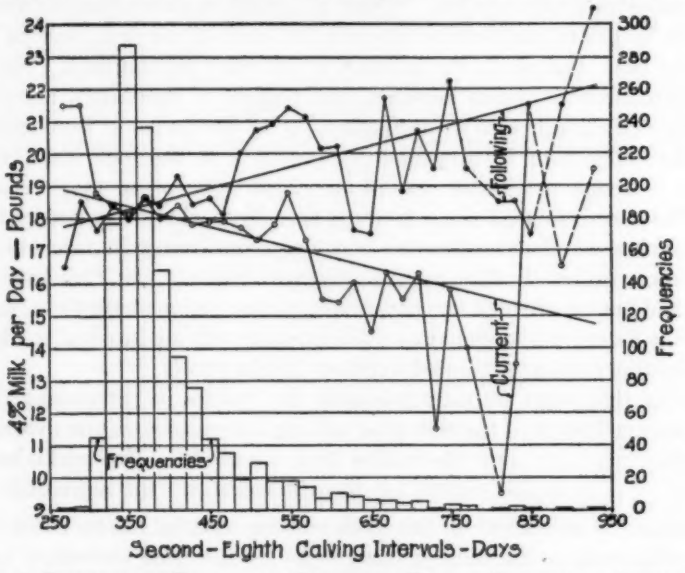

FIG. 1. SHOWING THE RELATION OF LENGTH OF CALVING INTERVAL TO AVERAGE YIELD OF 4 PER CENT MILK PER DAY OVER THE CURRENT CALVING INTERVAL AND OVER THE FOLLOWING CALVING INTERVAL, ALSO THE FREQUENCY DISTRIBUTION OF CALVING INTERVALS

Equations of fitted curves:

$$y = 20.61 - 0.00635\,x$$
$$y = 16.02 + 0.00652\,x$$

but one having a calving interval of one year, the other, of two years, it follows that the second lactation of one cow will begin at three years of age, the other, at four years of age. A similar condition will exist, of course, in subsequent calving intervals, but the effect on yield will be less marked. In dealing with $r_{I_n Y_{n+1}}$ it was deemed expedient, therefore, to exclude the first calving interval.

Where n takes successively values of two to eight inclusive, giving $186 \times 7 = 1302$ observations, the following correlations were found:

$$
\begin{aligned}
r_{I_n Y_n} &= -0.134 \pm 0.018 \\
r_{I_n Y_{n+1}} &= 0.142 \pm 0.018 \\
r_{I_n I_{n+1}} &= 0.088 \pm 0.019 \\
r_{Y_n Y_{n+1}} &= 0.527 \pm 0.014 \\
r_{I_n Y_n \cdot I_{n-1}} &= -0.148 \pm 0.018 \\
r_{I_n Y_{n+1} \cdot I_{n+1}} &= 0.156 \pm 0.018 \\
r_{I_n Y_{n+1} \cdot Y_n} &= 0.252 \pm 0.018
\end{aligned}
$$

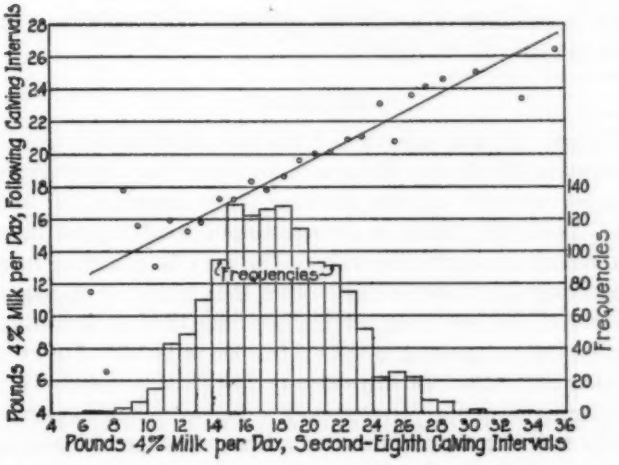

FIG. 2. SHOWING THE RELATION OF YIELD TO THE FOLLOWING YIELD, ALSO FREQUENCY DISTRIBUTION OF YIELDS, WHERE YIELD IS THE AVERAGE YIELD OF 4 PER CENT MILK OVER THE CALVING INTERVAL

Equation of fitted curve:

$$y = 9.39 + 0.510\,x$$

These correlations show that the length of calving interval is related to both the current yield and the following yield, and to about an equal degree. The observed regressions, treating the yield as dependent on calving interval, are shown in figure 1. The observations are still quite irregular, but the linear regres-

sions derived from the constants may be regarded as describing the trends satisfactorily. The slopes are practically equal and in opposite directions. The data in this form fully support Matson's point in that what is gained currently by a short calving interval is "paid for" in the next lactation with a fine exactitude.

There is apparent in figure 1 a tendency for the observed curves to parallel each other. This is easily explained as due to the fact that the two observations for each calving interval class represent the same group of cows. If a group average is high this lactation it will be high also the next lactation. The correlation $r_{Y_nY_{n+1}} = 0.527$ describes this characteristic, and simply indicates a certain degree of stability in productive ability. This feature is further shown graphically in figure 2.

DISCUSSION

The mean calving interval for the first nine lactations of these 186 cows is 401 days. Reference to figure one, however, shows the most common calving interval to be between 350 and 370 days. Evidently there is a pronounced tendency to have the cows freshen at yearly intervals. The very long calving intervals are probably due to some cause other than intent of management.

In case of the very long calving intervals there is undoubtedly a selection factor entering in with reference to yield. For example, a calving interval of 650 days means that the cow was not in calf a year after freshening. Unless such a cow were holding up exceptionally well in milk yield at this advanced stage of lactation she would be sold for beef. It seems to be clear, however, that if it is desirable to shift the season of calving, prolonging the calving interval up to 18 months need not adversely affect the average yield.

In connection with table 3 it may be permissible to mention a point somewhat apart from our subject. The point pertains to the suitability of the average yield over the calving interval as a measure of yield for biological study. The suitability of this measure as a matter of economics is evident. Is it equally suitable as a biological measure?

Bearing on this question table 4 is assembled from table 3 and from Gowen's (5) (6) work. Gowen's data are based on the records of Jersey cows in a single large herd and deal with the first eight months of lactation. His yield data represent actual milk production. The point of interest is as to the use of an absolute yield over a fixed portion of the lactation, and the use of an average figure over the entire lactation and dry periods. The higher correlations existing where a fixed portion of the lactation is used indicate that this is a more suitable measure of yield. The corre-

TABLE 4

Coefficients of correlation between successive lactations bearing on the suitability of different measures of yield for biological analysis

LACTATIONS CORRELATED	LINE*	COEFFICIENTS OF CORRELATION	
		Yield	Fat percentage
First and second	a	0.451 ±0.015	0.733 ±0.009
	b	0.599 ±0.046	0.711 ±0.036
Second and third	a	0.558 ±0.013	0.774 ±0.007
	b	0.779 ±0.028	0.687 ±0.038
Third and fourth	a	0.583 ±0.012	0.811 ±0.006
	b	0.621 ±0.044	0.699 ±0.037
Fourth and fifth	a	0.618 ±0.012	0.794 ±0.007
	b	0.666 ±0.040	0.576 ±0.048

* The coefficients in line a are from the present paper. Those in line b are from Gowen, table 53 (5), and table 49 (6).

lations for fat percentage show higher coefficients for the complete lactations of Langmack's Red Danish material than was found by Gowen for his eight months Jersey material.

SUMMARY

Records published by Langmack (3) are considered with respect to the effect of length of calving interval on the average milk yield per day over the calving interval. The present data are confined to 186 Red Danish cows whose records in each case start with the cow's first calf and continue uninterruptedly through the nine

following calvings, all ten calvings being normal, that is, no abortion. Yield is considered on an energy basis in terms of 4 per cent milk.

There is uniformly a negative correlation between calving interval and yield over the current calving interval, and a positive correlation between calving interval and yield over the following calving interval. Throughout, the relation is irregular and the correlation coefficients are small in value. When the several calving intervals (excluding the first because the influence of age on yield is most marked at this stage of life) are thrown together the correlation between calving interval and yield over the current interval is $r = -0.134 \pm 0.018$; that over the following interval, $r = 0.142 \pm 0.018$. Thus, while there is a small gain from a short calving interval in the current lactation it is very exactly lost in the following lactation.

The calving interval distribution shows a mean of 401 days, but with a pronounced mode between 350 and 370 days. If it should be desirable to shift the time of year of calving it appears that the calving interval may be prolonged to 18 months without adversely affecting the average yield per day, considered over both the current and following intervals.

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DIETARY FACTORS INFLUENCING CALCIUM ASSIMILATION*

XIV. THE INFLUENCE OF MINERAL ACIDS AND SUGAR ON THE CALCIUM METABOLISM OF MILKING COWS

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It is becoming more and more evident that the metabolism of calcium in the lactating cow follows a rather definite path. First, in the early period of lactation, *especially with high milk flow*, the calcium assimilation from the digestive tract is insufficient to meet the needs of mammary secretion and the skeleton is drawn upon, with a negative calcium balance resulting. Second, with progressive lactation and a falling milk production a point is reached where calcium equilibrium is established; that is, sufficient calcium is absorbed from the digestive tract to maintain the calcium level of the blood without drawing upon the skeletal tissue. Third, on still lower production, and especially when the animal is dry, the assimilation of calcium from the digestive tract is sufficient not only to meet the needs of either a low milk production or calcium maintenance, but actually to supply sufficient calcium for active storage in the skeleton. In other words, in the period of low milk production or when dry the depleted calcium reserves of the bones are restored and the skeleton of the animal again put back, in respect to its mineral content, to the same place it started at the beginning of lactation. The above outline seems to portray the general situation, which may be modified, but not wholly corrected, by the level of calcium in the diet. Most

* Received for publication January 2, 1931. Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

of the published evidence indicates that in feeding legume hays or timothy hays fortified with lime salts, the losses of calcium to the animal will be reduced as compared with timothy hay alone, but even under these conditions calcium equilibrium or storage may not generally be established in the period of high milk production. The excellent records recently published by Huffman and associates (1) on calcium balances of lactating cows over entire lactation periods is experimental evidence for the above picture.

Just what forces are operative in the release of calcium from the bones during the early part of lactation is unsolved; but it is evident that there is some active compensatory mechanism, supplementing the calcium derived from the digestive tract, with the bones as the source of the calcium needed. Apparently these negative calcium balances in the early stages of lactation are without disturbance to the normal physiological function of the animal up to a certain point; probably a danger point is not reached because of the constantly lowering milk secretion with progressive lactation. For a number of years it has been our aim to stop, if possible, these calcium losses in the early part of lactation, and our later work on calcium metabolism of milking cows has been directed to that purpose.

In all previous work on this problem carried out at this station attempts have been made to influence favorably the assimilation of calcium in dairy cows during the early part of their lactation period through the use of such factors as vitamin D and calcium levels. In general it has been our experience that upon dry rations, with corn silage, the addition of calcium salts has not resulted in the production of positive calcium balances, although the balances may have been somewhat improved (2). This is in agreement with the work of Forbes and others (3). Ellenberger and Newlander (4) and Huffman and associates (1) report a better utilization of calcium salts added to the winter ration of dairy cows than observed by most of the other investigators in this field. Further, the increase in the supply of vitamin D, either in the form of cod liver oil, direct irradiation of the animal, or irradiated yeast, has also failed to convert negative calcium

balances into positive calcium balances during the early period of lactation. On the other hand, there has been observed at this station, as well as by other investigators, that when the level of calcium is sufficiently high *fresh green plant tissue* favorably influences calcium assimilation. For example, in 1927 (5) in an experiment on calcium level and sunlight as effecting calcium equilibrium in milking cows it was found that these animals in the flush of milk production and yielding 50 pounds of milk per day could be kept in positive calcium balance when the ration consisted of about 40 pounds of green grass, 30 pounds of corn silage, and a grain mixture of 59 parts of yellow corn, 25 of wheat bran, 15 of oil meal, 1 of common salt, and a calcium salt addition. It was believed at that time, and subsequent to those experiments, that the factor favorably influencing the assimilation of calcium was the vitamin D of the green grasses. However, the fact that irradiated yeast (6) fails to influence favorably calcium assimilation in milking cows and that green grass does not contain much vitamin D excludes vitamin D as the dominating factor concerned in the green grass experiments, and makes it appear that there are other factors in the green plant tissue that favorably influenced calcium assimilation.

For a working plan we postulated the hypothesis that probably in the green grasses there were fairly high levels of fermentable sugars; that these sugars either by conversion into acids along the tract aided calcium assimilation by virtue of holding the calcium in solution, or that the sugars themselves increased the solubility of the calcium salts. It is well known that sugars may have the latter influence. We tested this theory by adding to the ration specific amounts of mineral acids such as hydrochloric acid or definite quantities of Cerelose, a glucose preparation made by the Corn Products Refining Company.

EXPERIMENTAL

Experiment 1

For our first experiment three Holstein cows in the early part of lactation were selected. They were fresh but a few weeks

and gave between 40 and 45 pounds of milk per day. A fore-period of four weeks was run with a complete calcium balance on the animals. The ration consisted of 10 pounds of chopped alfalfa hay, 25 pounds of corn silage, and a grain mixture made up of 59 parts of yellow corn, 25 of wheat bran, 15 of oil meal, and 1 of salt. The grain was fed at the rate of 1 pound for about 3 pounds of milk produced.

In the second period of four weeks the same ration was continued, but to the alfalfa hay there were added daily either 115 or 230 cc. of 40 per cent hydrochloric acid—which would be equivalent to the addition of 46 or 92 grams of hydrochloric acid respectively. The addition of the acid was carried out as follows: The chopped alfalfa hay was placed in a wooden tub; the acid was diluted with water; and then the solution poured over the hay and stirred in. This mixture was prepared once daily for both the morning and evening feeding, allowing it to stand over night. The amount of water used to dilute the hydrochloric acid depended upon the amount necessary to moisten the hay.

This addition of the acid to the hay in no way disturbed the appetites of the animals. They consumed the ration with avidity and completeness. Two of the cows, nos. 2 and 3, in the second period of the experiment received the acid. The third cow, no. 1, received the basal ration fed in the fore-period, but with the substitution in the second period of 3 pounds of cerelose for 3 pounds of her grain ration; because of the reduction in the protein due to this displacement 0.6 pound of crude casein was added to her daily ration to compensate for the reduced protein.

The actual experiment began on October 24. Cow 1 was fresh October 4; cow 2, October 1; and cow 3, October 2. The udder of cow 3 became infected and it was necessary to displace her by another animal, which received the basal ration for but two weeks instead of four weeks, and the ration containing the acid for three weeks instead of four weeks. None of the cows were bred during the time of the experiment. Consumption of the ration was complete throughout the entire period.

TABLE 1

Cow 1—Ca balance and milk production—influence of sugar on calcium assimilation

WEEK	CaO IN FECES	CaO IN MILK	CaO IN URINE	TOTAL CaO EXCRETED	TOTAL CaO INTAKE	Ca BALANCE	MILK PER WEEK
			Period I—basal ration				
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*pounds*
1	573.85	225.45	14.37	813.67	819.96	+6.29	302.8
2	595.19	224.11	18.27	837.57	819.96	−17.61	301.0
3	644.33	205.55	14.18	864.06	819.96	−44.10	292.1
4	627.75	206.76	17.71	852.22	819.96	−32.26	279.4
Total....	2,441.12		64.53			−87.68	
			Period II—basal ration + sugar (3 pounds daily)				
5	565.49	203.72	9.73	778.94	806.63	+27.69	284.0
6	612.06	202.81	15.57	830.44	806.63	−23.81	283.6
7	558.09	196.81	13.34	768.24	806.63	+38.39	279.8
8	556.25	203.72	20.54	780.51	806.63	+26.12	284.0
Total....	2,291.89		59.18			+68.39	

TABLE 2

Cow 2—Ca balance and milk production—influence of acid on calcium assimilation

WEEK	CaO IN FECES	CaO IN MILK	CaO IN URINE	TOTAL CaO EXCRETED	TOTAL CaO INTAKE	Ca BALANCE	MILK PER WEEK
			Period I—basal ration				
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*pounds*
1	527.87	222.25	8.24	758.36	825.21	+66.85	313.8
2	516.85	229.98	4.49	751.32	825.21	+73.89	318.6
3	604.78	221.23	6.78	832.79	825.21	−7.58	318.5
4	653.18	228.77	10.34	892.29	825.21	−67.08	325.1
Total....	2,302.68					+66.08	
			Period II—basal ration + 115 cc. strong HCl (daily for two weeks)				
5	562.18	228.27	10.23	800.68	825.21	+24.53	322.3
6	545.01	231.38	15.94	792.33	825.21	+32.88	328.8
			Period III—basal ration + 230 cc. strong HCl (daily for two weeks)				
7	547.09	218.82	56.43	822.34	825.21	+2.87	319.2
8	546.48	221.60	74.37	842.45	825.21	−17.24	314.9
Total....	2,200.76					+43.04	

TABLE 3

Cow 3—Ca balance and milk production—influence of acid on calcium assimilation

WEEK	CaO IN FECES	CaO IN MILK	CaO IN URINE	TOTAL CaO EXCRETED	TOTAL CaO INTAKE	Ca BALANCE	MILK PER WEEK
Period I—basal ration							
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*pounds*
1	593.28	221.40	9.91	824.59	825.21	+0.62	302.9
2	593.71	213.13	10.26	817.10	825.21	+8.11	293.4
Total.....	1,186.99					+8.73	
Period II—basal ration + 115 cc. strong HCl (daily for two weeks)							
3	632.40	211.87	8.62	859.89	825.21	−34.68	293.5
4	573.70	206.80	21.78	802.28	825–21	+22.93	288.3
5	582.66	199.71	29.33	811.70	825.21	+13.51	283.8
⅗ Total....	1,192.50					+1.76	

TABLE 4

Weekly titrations of milk, urine, and feces

WEEK	pH OF MILK—BASAL RATION			pH OF URINE—BASAL RATION			pH OF FECES—BASAL RATION		
	Cow 1	Cow 2	Cow 3	Cow 1	Cow 2	Cow 3	Cow 1	Cow 2	Cow 3
1	6.95	6.80	6.90	8.25	9.80	8.80	7.12	7.13	7.38
2	6.80	6.95	6.72	7.85	9.80	9.10	8.40	8.77	7.15
3	6.75	6.85	6.70	9.25	9.80	7.80	8.25	8.45	7.02
4	6.95	6.85	6.72	9.60	9.80	8.15	7.95	8.05	6.67
	+ Sugar	+ Acid	+ Acid	+ Sugar	+ Acid	+ Acid	+ Sugar	+ Acid	+ Acid
5	6.83	6.92	6.75	9.05	8.35	7.75	7.20	7.30	7.03
6	6.80	6.87		8.90	8.18		6.85	7.90	
7	6.67	6.85		9.30	7.95		7.05	6.95	
8	6.95	6.87		9.15	7.50		6.80	6.53	

pH of urine—daily for weeks 7 and 8 { cow 2—230 cc. acid daily; cow 3—115 cc. acid daily }

COW	DECEMBER 6	DECEMBER 9	DECEMBER 10	DECEMBER 11	DECEMBER 12	DECEMBER 13	DECEMBER 14	DECEMBER 15	DECEMBER 16	DECEMBER 17	DECEMBER 18
2	6.75	7.95	6.42	7.57	8.32	6.67	7.14	6.95	7.50	7.32	7.53
3	7.65	8.15	7.95	7.75	7.76	7.97	7.85	7.67	7.75	7.87	7.75

In addition to the calcium balances secured, complete data were also secured at definite periods of the experiment upon the calcium and phosphorus content of the blood, upon the reaction of the milk, and upon the reaction of the urine and feces. By these determinations, especially the acid determination on the urine, information was secured as to the influence of the acid upon the general reaction of the excretory products. The pH was determined with the quinhydrone electrode. The data secured in this first experiment are brought together in tables 1, 2, 3, 4 and 5.

In the case of cow 1 there was a total negative calcium balance during the fore period of 87.68 grams of calcium oxide with 2441 grams of calcium oxide in the feces. In the period where she

TABLE 5

Blood Ca and phosphorus

Milligrams per 100 cc. serum

	cow 1		cow 2		cow 3	
	Ca	Inorganic P	Ca	Inorganic P	Ca	Inorganic P
End of first period	10.44	6.66	9.58	5.03	10.91	5.03
Middle of second period	11.25	6.02	10.35	6.36		
End of second period	10.24	6.19	9.88	5.00	10.74	4.73

received the sugar a *positive* calcium balance of 68.39 grams prevailed and the calcium oxide in the feces amounted to 2291 grams or a reduction of about 150 grams. The data secured with this animal would indicate that there had been some favorable influence from the use of the sugar on the calcium assimilation.

In the case of the two cows receiving the acid, no. 2 in the fore-period showed a positive calcium balance of 66 and 2302 grams of calcium oxide in the feces. In the acid ration period for the first two weeks she received but 115 cc. daily, while in the last two weeks she received 230 cc. daily. The effect of the acid was to increase the calcium excretion in the urine—which was to be expected. Her balance for the four weeks of acid feeding was

positive by 43 grams, with 2200 grams of calcium oxide in the feces, or a reduction of about 100 grams over the fore-period. The fact that there was a marked increase in calcium excretion in the urine where the high amount of acid was fed, disturbed of course, a more favorable calcium balance of the animal as a whole. The calcium oxide in the urine rose from about 10 grams per week to 75 grams per week; and although there appeared to be a favorable influence of the acid on the assimilation of the calcium from the gut, this increased excretion in the urine gave a net result to the animal a little less favorable than where the acid was not added.

Practically the same situation occurred in the case of cow 3, although the periods for observation were shorter than in the case of cow 2. She showed a positive calcium balance during the first two weeks of the experiment on the basal ration. During the period where she received 115 cc. of acid per day the calcium in the urine increased. It rose from 8 grams per week to about 29 grams per week; but the net result of the addition of the acid was nevertheless a positive balance, although less positive than in the period where no acid was added.

These results from the addition of mineral acid would indicate some favorable influence upon calcium assimilation, but the necessity of using some of the base for the neutralization of the acid as it is excreted into the urine shows no favorable influence to the animal as a whole from such additions; whether an organic acid such as lactic or acetic would be more favorable in its effect through its partial or complete oxidation and consequent reduction of the losses of calcium through the urine is altogether possible.

The pH of the milk was in no way influenced by the acid additions. This is in conformity with past experiments at this station (7), where sulphuric acid has been added to the rations of dairy cows without influencing whatever the reaction of the milk.

The reaction of the urine was distinctly influenced by the acid additions. During the pre-acid period in the case of cow 2 the urine had a pH of 9.8. This was reduced to 8.2 during the

period of low acid feeding and to 7.5 during the period of high acid feeding. In fact, during the last two weeks the daily titration of the urine varied in its pH, sometimes reaching as low as 6.4, but the average for the period was still on the alkaline side.

In the case of cow 1, where the sugar was added, there was no change in the pH of the urine. It remained on the alkaline side with a pH of 8 to 9 during the entire period.

The reaction of the feces was also apparently influenced by the acid treatments. During the fore-period the pH of the feces varied from 7 to 8; but during the acid feeding, and especially during the high acid treatment of the last two weeks, the pH of the feces decreased to 6.5. There seemed to be a somewhat similar tendency under the conditions of sugar feeding, but it was only slight.

The calcium and phosphorus content of the blood did not vary in the two periods.

Experiment 2

Because of the encouraging results from the use of sugar (cerelose) as a factor influencing calcium assimilation secured in experiment 1, in the spring of 1930 we set up another experiment which was limited wholly to testing further the influence of sugar on calcium assimilation in fresh milking cows.

Two Holsteins and one Ayrshire were chosen for this work. The experiment began on April 3. The cows had freshened as follows: No. 1, a Holstein, March 22; no. 2, a Holstein, February 17; no. 3, an Ayrshire, February 5. They were cows of four to six years of age and very quiet in the metabolism stalls. Their production was not high. The Holsteins produced from 35 to 40 pounds of milk per day, while the Ayrshire was producing from 45 to 50 pounds. Because of the somewhat lowered milk production and the necessity of giving the sugar theory a more crucial test the two Holsteins were placed upon a ration of timothy hay, corn silage, and grains. They were fed 10 pounds of timothy hay, 25 pounds of corn silage, and a grain mixture consisting of 64 parts of yellow corn, 10 of oil meal, 25 of wheat bran, and 1 of common salt. The Ayrshire was placed upon an

TABLE 6

Cow 4—Ca balance and milk production

(Timothy hay)

WEEK	CaO IN FECES	CaO IN MILK	CaO IN URINE	TOTAL CaO EXCRETED	TOTAL CaO INTAKE	Ca BALANCE	MILK PER WEEK
	Period I—basal ration						
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*pounds*
1	205.14	183.30	6.05	394.49	247.82	−146.72	244.7
2	185.26	185.70	9.27	380.23	247.82	−132.41	239.2
3	210.33	172.72	6.52	389.57	247.82	−141.75	236.3
4	230.11	161.59	6.92	398.62	247.82	−150.80	226.7
Total....	830						
	Period II—basal ration + 3 pounds sugar daily						
5	239.22	162.85	9.51	411.58	257.16	−154.42	225.6
6	220.70	164.07	6.23	391.00	257.16	−133.84	217.7
7	216.11	156.07	4.89	377.07	257.16	−119.91	216.2
8	222.71	154.29	4.46	381.46	257.16	−124.30	212.4
Total....	898						

TABLE 7

Cow 5—Ca balance and milk production

(Timothy hay)

WEEK	CaO IN FECES	CaO IN MILK	CaO IN URINE	TOTAL CaO EXCRETED	TOTAL CaO INTAKE	Ca BALANCE	MILK PER WEEK
	Period I—basal ration						
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*pounds*
1	171.82	230.28	7.30	409.40	247.82	−161.58	294.9
2	174.16	198.70	6.96	379.82	247.82	−132.00	277.0
3	228.97	177.63	6.85	413.45	247.82	−165.63	257.4
Total.....	574.95						
	Period II—basal ration + 3 pounds sugar daily						
4	243.85	175.67	6.08	425.60	257.16	−168.44	252.9
5	253.21	171.57	4.79	429.57	257.16	−172.41	247.0
6	213.32	162.02	11.68	387.02	257.16	−129.86	227.3
7	235.59	151.75	14.24	401.58	257.16	−144.42	219.9
¾ Total....	702.00						

alfalfa, corn silage, grain ration with the protein content of the grain mixture much less in order to compensate for the higher protein of the alfalfa hay. Her grain mixture consisted of 69 of yellow corn, 5 of oil meal, 25 of wheat bran, and 1 of common salt.

It was planned to run the basal rations for a preliminary period of four weeks. In the second four weeks there was a displacement of 3 pounds of grain for 3 pounds of sugar (cerelose) plus

TABLE 8

Cow 6—Ca balance and milk production

(Alfalfa hay)

WEEK	CaO IN FECES	CaO IN MILK	CaO IN URINE	TOTAL CaO EXCRETED	TOTAL CaO INTAKE	Ca BALANCE	MILK PER WEEK
			Period I—basal ration				
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*pounds*
1	657.72	227.53	17.63	902.88	1,069.62	+166.44	331.9
2	671.19	248.39	25.13	944.71	1,069.62	+124.91	333.6
3	667.31	228.37	19.60	915.28	1,069.62	+154.34	320.4
4	695.08	215.19	12.74	923.01	1,069.62	+146.61	309.8
Total....	2,691						
			Period II—basal ration + 3 pounds sugar daily				
5	729.19	224.81	7.88	961.88	1,081.82	+119.94	315.4
6	797.77	225.72	7.65	1,031.14	1,081.82	+50.86	312.7
7	805.26	232.22	5.57	1,043.05	1,081.82	+38.77	310.0
8	735.13	236.78	6.68	978.59	1,081.82	+103.21	305.0
Total....	3,067						

0.6 pound of crude casein. Records were kept of calcium balance, calcium and phosphorus content of the blood, reaction of the urine, milk, and feces. The cows were quiet and contented throughout the entire experiment. They were not bred, and their consumption of the rations was complete during the entire eight weeks. The amount of grain each one was fed depended upon the milk produced and was approximately 1 pound of grain for 3 pounds of milk. The records of the calcium balances are displayed in tables 6, 7, and 8.

With cow 1 (timothy hay) a negative calcium balance prevailed throughout the pre-sugar and the sugar period. The degree of loss of calcium was not reduced by the ingestion of 3 pounds of sugar daily. Eight hundred and thirty grams of calcium oxide were excreted in the feces during the pre-sugar period and 898 grams during the sugar period. In the case of cow 2 (timothy hay) results similar to those secured with cow 1 prevailed. In the three weeks of feeding the ration without sugar 574 grams of calcium oxide appeared in the feces, while in the first three weeks of the sugar period 709 grams of calcium oxide were excreted in the feces. In the case of cow 3 (alfalfa hay), where the ingestion of calcium oxide was approximately four times that of the timothy hay ration, positive balances appeared throughout the duration of the experiment. However, during the sugar feeding the amount of lime excreted into the gut was larger than the amount excreted during the period where no sugar was fed.

These experiments indicate that there was no positive influence on calcium assimilation resulting from mixing sugar (cerelose) with the ration; merely mixing sugar with the ration may not imitate the conditions that exist in a green plant cell, and until such imitations can be made one cannot positively know whether there is any value in the idea of the possible influence of sugar (cerelose) upon the assimilation of calcium. Recently Hunt and co-workers (8) have proposed the idea that the superior assimilation of calcium from green plant tissue as compared with dried plant tissue rests upon a more perfect dispersion of calcium in the green cell than in the dry cell, and that in this more highly dispersed condition the calcium is better assimilated. In opposition to that view, however, the fact must be recorded that Forbes (3) observed no beneficial effect on calcium assimilation when he added such soluble salts as calcium lactate or calcium chloride to a dry winter ration. It would seem that calcium salts as chlorides or lactates should be as readily absorbed as calcium salts in a dispersed state in the green plant cell.

In table 9 the record of the reaction of the milk, urine, and feces are given. It will be seen from these records that the

ingestion of sugar had no effect whatever upon the reaction of milk. Further the reaction of the urine remained alkaline in the pre-sugar period as well as in the sugar period, and there was little if any influence from the sugar upon this reaction. The record of the reaction of the feces also gives no reason for believing that the sugar had influenced the reaction of the bowel excretions.

TABLE 9

Weekly titrations of milk, urine, and feces

WEEK	pH OF MILK—BASAL RATION			pH OF URINE—BASAL RATION			pH OF FECES—BASAL RATION		
	Cow 1	Cow 2	Cow 3	Cow 1	Cow 2	Cow 3	Cow 1	Cow 2	Cow 3
1	6.80	6.65	6.81	8.15	8.52	8.20	5.88	6.01	7.05
2	6.84	6.75	6.75	8.68	8.20	8.34	6.15	5.95	7.10
3	6.90	6.67	6.85	8.00	8.15	8.55	5.75	6.15	6.15
4	6.87	6.65	6.75	8.50	8.35	8.03	6.05	5.95	6.85
	+ Sugar	+ Acid	+ Acid	+ Sugar	+ Acid	+ Acid	+ Sugar	+ Acid	+ Acid
5	6.80	6.75	6.82	8.65	7.85	8.12	5.67	5.98	6.82
6	6.82	6.69	6.97	7.30	8.03	8.00	6.25	6.23	6.75
7	6.75	6.75	6.83	7.51	7.10	8.37	6.20	6.20	6.62
8	6.84		6.90	8.35		8.12	5.85		6.65

TABLE 10

Blood Ca and phosphorus

Milligrams per 100 cc. serum

	cow 1		cow 2		cow 3	
	Ca	Inorganic P	Ca	Inorganic P	Ca	Inorganic P
End of pre-sugar period	11.76	7.39	10.78	7.05	10.88	6.65
End of sugar period	10.00	5.39	10.83	5.35	10.02	5.00

In table 10 is recorded the total calcium and inorganic phosphorus of the blood serum. It will be noticed that these results are very uniform in both periods and give no indication that there had been an influence on the calcium and inorganic phosphorus levels of the blood through the ingestion of sugar (cerelose).

While our data as a whole show no positive or consistent influence of sugar (cerelose) on the calcium assimilation in dairy

cows, it should be pointed out that Robinson, Huffman, and Mason (9) did observe a favorable influence on calcium absorption in calves through the use of lactose. Also, in rats Bergeim (10) records a better utilization of calcium when the diet contained lactose than when the sugar was glucose, sucrose, maltose, or starch.

SUMMARY

In this paper data are presented on the influence of cerelose (glucose) and mineral acid (HCl) on the absorption of calcium by the milking cow.

1. There was no consistent and favorable influence on calcium assimilation in dairy cows through the daily addition of 3 pounds of cerelose (glucose) to a standard ration of hay, silage, cereal grains, and cereal grain concentrates.

2. The daily ingestion of 115 or 230 cc. of 40 per cent HCl increased the calcium excretion in the urine with a greater net loss of calcium to the animal than when no acid was fed. The calcium absorption from the intestine was slightly improved through the use of mineral acid.

3. What factor in green plant tissue operates in securing for the cow a better utilization of the calcium in the green plant tissue or added calcium salts is not solved by the technique used in the above experiments.

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CALCIUM ASSIMILATION AS INDICATED BY BONE ANALYSIS IN LONG-TIME EXPERIMENTS*

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INTRODUCTION

The assimilation of calcium by dairy cows appears to be influenced by a number of factors. These are, the general character of the food, the quality of the roughage as influenced by the conditions of curing, the nutritive condition or individuality of the animal, the proportions of calcium and phosphorus in the diet, the quantity of calcium in the diet, and the milk yield.

Investigations of the question of calcium assimilation by dairy cows have been carried out almost entirely by means of balance experiments in which the intake and outgo of this element have been determined for varying periods of time and under various conditions of feeding. It seems desirable that the results of such experiments be supplemented by estimations of the assimilation of calcium by animals maintained under herd conditions and on a single dietary regimé for long periods of time.

It is the purpose of this paper to present the results obtained: (*a*) From calculations of the calcium assimilations of four cows fed continuously for periods of fourteen to thirty-two months on the same diet; (*b*) from the correlation of the figures for assimilation with the results of analyses and weights of bones obtained from one of these animals and from two other animals which had been on alfalfa hay for some time before death.

In reports of balance experiments carried out at this station, calcium assimilation for an experimental period has been defined (1) as the calcium contained in the food consumed minus the calcium contained in the urine and feces excreted during that period, due account being taken of intestinal lag. This value affords a very close approximation of the net intestinal absorp-

* Received for publication January 12, 1931.

tion. Percentage assimilation is this difference expressed as per cent of the intake.

EXPERIMENTAL

Long-continued feeding experiments at this station afforded the opportunity of studying calcium assimilation under practical conditions.

Four cows, three purebred Jerseys and one grade Guernsey, were fed rations consisting of timothy hay, a grain mixture and corn silage[1] for periods of fourteen to thirty-two months. Records were kept of the food consumed, the milk yielded, the weights of calves dropped, and the body weights of the animals.

The hay fed was certainly not better than average in quality and probably all of it would be classed as U. S. No. 2, some being a low-grade No. 2. The grain mixture, No. 55, was composed of corn meal 30, wheat bran 20, cottonseed meal 25, linseed meal 25, and salt 1 parts by weight.

For the whole period of timothy feeding, Cow 429 consumed 107.8 per cent of Savage requirements for total digestible nutrients; Cow 54, 112.6 per cent; Cow 456, 104.8 per cent; and Cow 450, 97.2 per cent. All four animals consumed considerably more digestible protein than required by the Savage standard.

No balances were determined on these four animals at any time during the experiment and the method of calculating the percentage calcium assimilation was as follows:

The calcium intake was determined from the percentages of calcium in the feeds and the quantities of the feeds consumed during the whole period; the outgo of calcium was calculated from the percentage of this element in the milk and from the total milk yield for the period and from the calcium contents and weights of the calves dropped during this time. The percentage assimilation was then calculated in accordance with the following equation:

$$\text{Percentage assimilation} = \frac{\text{Ca in milk} + \text{Ca in calves}}{\text{Ca in food}} \times 100$$

[1] Cow 450 received no silage.

TABLE 1

Composition of bones

BONE	WEIGHT OF BONE*			ASH			CALCIUM			PHOSPHORUS			ORGANIC MATTER			PROTEIN†			WATER		
	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17
	T.‡	A.‡	A.	T.	A.	A.	T.	A.	A.	T.	A.	A.	T.	A.	A.	T.	A.	A.	T.	A.	A.
Dry basis																					
	grams	*grams*	*grams*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Radius and ulna				53.2	52.2	58.6	20.8	20.2	22.7	9.51	9.37	10.44	46.8	47.8	41.4	25.0	23.3	24.9			
Tibia				50.0	47.3	55.8	19.5	18.5	21.5	8.97	8.50	9.94	50.0	52.7	44.2	22.9	19.9	23.4			
Metacarpus				56.9	56.1	61.3	22.2	21.6	23.7	10.07	10.06	10.90	43.1	43.9	38.7	25.9	24.9	25.4			
Metatarsus				58.4	56.6	62.8	22.9	21.8	24.3	10.43	10.11	11.18	41.6	43.4	37.2	26.5	25.3	26.2			
10th and 11th ribs				56.8	56.0	62.4	22.3	21.6	24.1	10.04	10.06	11.10	43.2	44.0	37.6	30.9	28.2	30.2			
Original moisture basis																					
Radius and ulna	722	902	640	46.2	46.3	46.8	18.1	18.0	18.2	8.26	8.31	8.34	40.7	42.4	33.1	21.7	20.7	19.9	13.1	11.3	20.1
Tibia	816	1,016‖	776	43.8	42.1	44.9	17.1	16.4	17.3	7.87	7.56	7.99	43.9	46.9	35.5	20.1	17.7	18.8	12.3	11.0	19.6
Metacarpus	260	340‖	259	50.2	49.6	50.0	19.6	19.1	19.4	8.88	8.89	8.89	38.0	38.8	31.6	22.8	22.0	20.7	11.8	11.6	18.4
Metatarsus	333	530‖	342	51.6	49.2	52.8	20.2	19.0	20.4	9.22	8.80	9.39	36.8	37.8	31.2	23.4	22.0	22.0	11.6	13.0	16.0
10th and 11th ribs	168§	203§	176§	46.2	47.1	51.4	18.2	18.2	19.8	8.16	8.45	9.14	35.0	37.0	30.9	25.2	23.7	24.9	18.7	16.0	17.7

* Average weight of the bones from both sides of the animal. Only one bone analyzed.

† Nitrogen × 6.25.

‡ T. = timothy-fed; A. = alfalfa-fed.

§ Average of weights of 10th, 11th, 12th and 13th ribs.

‖ Weight of bone from one side of animal only.

This method of determining the calcium assimilation presupposes no net change in the calcium stores of the body for the period of feeding. In order to get some idea of the extent to which the bones had been depleted of calcium after a long period of low-calcium (timothy hay) feeding, bones were obtained from one of these cows (No. 429)[2] which had been on timothy for thirty months. These bones were weighed and analyzed and the results were compared to weights and analyses of the same bones of two other animals which had been on alfalfa for some time before slaughter. The results of these analyses are given in table 1. Weights and analyses of separate bones are given and figures for phosphorus and protein are included. Cow 429, the timothy-fed animal, has been designated in the table by T; Cows 90 and 17, the alfalfa-fed animals, by A.

METHODS OF ANALYSIS

The bones were removed from the animal and cleaned superficially of attached tissue. They were then placed in a well-cooled refrigerator and remained there until taken out one at a time for thorough removal of tendons and remaining tissue. Much work was required to render the bones practically free of tissue and the small amount finally remaining could have had no noticeable effect upon the results of the analyses. After being cleaned they were returned immediately to the refrigerator and were kept there until weighed. The bones were always weighed after being in the refrigerator for some time and before weighing were allowed to come to about room temperature. Immediately after weighing they were shredded in a bone shredder and two large samples were weighed into 6-inch evaporating dishes. In most cases the two samples constituted practically the whole

[2] The death of Cow 429 occurred June 12, 1927. She had calved April 17, 1927, and yielded liberal amounts of milk for about six weeks. On May 30, 1927, she began to go off feed and down in her milk, and, in the course of a day or two, her milk yield and feed consumption were reduced to almost nothing and remained so until her death. On autopsy, she was found to have a rather general purulent inflammation, affecting the left lung, heart, diaphragm, liver, and mammary gland. (Quoted from an unpublished paper by E. B. Meigs on the performance of some animals on long time experiments.)

amount of shredded bone, and in all cases over half of it. The samples were dried for two or three days on a steam-heated sand bath; they were then allowed to come to an air-dried condition, ground finely in a small mill, placed in paraffin-sealed glass containers, and stored in the refrigerator until analysis was made. The bone prepared in this manner remains in excellent condition for quite a long period of time. Measurements of length, marrow cavity, etc., were made on some of the bones and in doing so change in moisture content was carefully determined and was corrected for in computing results of analyses.

Ash. Ash was determined by heating approximately 10-gram samples in small porcelain evaporating dishes in an electric muffle furnace at 1200°F. until constant weight was obtained. The ash so obtained was white or very light gray in color. During the ashing process the samples were frequently removed from the furnace and stirred with a small glass rod.

Calcium. The evaporating dish containing the ash was immersed in a solution of hydrochloric acid in a large beaker, warmed to hasten solution, cooled and made to a large volume (2000 cc.). Calcium was determined on aliquots of this solution by the method outlined in previous publications from this laboratory (2) (3), except that hydrochloric acid was displaced completely by sulphuric in the solution to be precipitated. This modification has been in use for a number of years at this laboratory and has the advantage of removing the possibility of interference of chlorides in the titration of the oxalate with $KMnO_4$.

Phosphorus. Phosphorus was determined as magnesium pyrophosphate by the method outlined in the article referred to above (2). Approximately 5-gram samples of bone were ashed in the wet way and diluted with water to about half the volume to which they were to be made. The suspended calcium sulphate was filtered off and washed, and the combined filtrate and washings were brought to 500 cc. in a volumetric flask. Aliquot portions of this solution were used for analysis.

Nitrogen. Nitrogen was determined on 2 to 4 grams of bone by the Kjeldahl-Gunning-Arnold official method (4).

Protein. Protein was calculated by multiplying the nitrogen by 6.25. This factor is sufficiently close for the purposes of this paper.

Organic matter. Organic matter was calculated by subtracting the per cent ash in the water-free bone from 100.

TABLE 2

Average bone analyses

	DRY BASIS						ORIGINAL MOISTURE BASIS					
	Long bones			Ribs			Long bones			Ribs		
	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17
	T.*	A.*	A.	T.	A.	A.	T.	A.	A.	T.	A.	A.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Ash	54.6	53.0	59.6	56.8	56.0	62.4	48.0	46.8	48.6	46.2	47.1	51.4
Calcium	21.4	20.5	23.1	22.3	21.6	24.1	18.8	18.1	18.8	18.2	18.2	19.8
Organic matter	45.4	47.0	40.4	43.2	44.0	37.6	39.8	41.5	32.8	35.0	37.0	30.9

* T. = timothy-fed; A. = alfalfa-fed.

TABLE 3

Histories of cows

COW NUMBER	AGE AT DEATH	TIME ON RATIONS	TOTAL MILK YIELD	DRY FOR:
	years	*months*	*kgm.*	*months*
429*	9	30	8,260	6½
90†	5¾	34	5,613	4
17‡	16	32½	4,137	8

* At death had completed first one and one-half months of a lactation.

† Slaughtered at end of nine months of a lactation; bred one month before slaughter.

‡ Dry for four to five months before slaughter; not pregnant.

Water. Water was determined by heating at 70°C. in vacuo (20 to 30 mm.) to constant weight.

In table 2 are given average results of analyses of long bones and of ribs and in table 3 some facts in the histories of these animals. Although moisture changes in handling the bones were determined and controlled as carefully as possible throughout,

the values on the dry basis are to be regarded, perhaps, as the more accurate of the two sets of figures in tables 1 and 2.

In the last thirty months of her life Cow 429, a purebred Jersey, was on timothy hay for this entire period. Of the last thirty-four months of her life Cow 90, a grade Guernsey, was on alfalfa for the first ten, timothy for the next fifteen, and on alfalfa for the last nine. Cow 17, a grade Jersey, was, during the last thirty-two and one-half months of life, on alfalfa for the first twenty-eight and one-half, on timothy for the next one and one-half, and on alfalfa for the last two and one-half. Cow 429 at death had completed the first one and one-half months of a lactation, No. 90 at slaughter had completed nine months of a lactation, and No. 17 was dry for from four to five months before slaughter.

TABLE 4

Bone weights as percentages of body weights

	cow 429	cow 90	cow 17
	per cent	*per cent*	*per cent*
Long bones	0.491	0.576	0.545
Ribs	0.155	0.168	0.191

From table 3 it is seen that No. 429 gave much more milk during this time than either of the alfalfa cows.

The figures (table 2) for bone analyses show throughout that there is little difference in the content of ash, calcium, and organic matter in the bones of No. 429, the timothy-fed cow, and No. 90, the alfalfa-fed cow, the former having, if anything, slightly higher values for ash and calium. On the dry basis, No. 17, the other alfalfa-fed cow, is distinctly higher in ash and calcium and lower in organic matter than are Nos. 429 and 90. On the original moisture basis, in the case of the long bones No. 17 exhibits about the same content of ash and calcium, this being accouted for by the higher water content of the long bones of this animal. The figures for the ribs of this cow are higher due to the water content being about the same as the other two cows. Cow 17 was, however, quite an old animal and it is well known that the mineral content of the bones increases somewhat in old age.

The results show that the long period of low-calcium feeding on timothy hay has brought about little if any change in the relative proportions of ash, calcium, phosphorus, and organic matter in the bones.

It may be supposed that the ratio of bone weight to body weight would give some indication of the loss of calcium from the skeleton, on the theory that the entire bone material disappears upon depletion of the minerals. The ratios of bone weight to body weight have, therefore, been calculated and are given as percentages in table 4. The figures for the long bones were obtained by dividing the sum of the average weights of the two metacarpi, metatarsi, radii and ulnae, and tibiae by the body weights and those for the ribs were obtained in a similar manner from the weights of the 10th, 11th, 12th, and 13thribs. The figure for the body weight of Cow 429 used in this calculation was obtained by averaging the monthly weights of the entire period on timothy hay, eliminating the four months before and the two months after calving. The body weight of Cow 90 was obtained in a similar manner from the monthly weights for two years previous to slaughter. That of Cow 17 was calculated from the first twenty-four of the thirty months preceding slaughter; these earlier monthly weights were used because it was noted that the body weights of the six or seven months immediately preceding death were low. If these low weights had been used in the calculations, the values of the ratios given in table 4 would have been increased.

The values of the ratio of bone weight to body weight in the case of Cow 429, the timothy-fed animal, are seen to be 10 to 20 per cent lower than those of the two alfalfa-fed cows. Considered on the basis of the theory upon which these figures have been calculated, these values suggest that the skeleton of No. 429 was depleted of calcium and of other inorganic elements to the extent of 10 to 20 per cent as a result of her long period on the timothy hay ration.

In table 5 are given the percentages of calcium assimilation of the four cows which were on the timothy rations for long periods. The last two columns show the number of lactations that fell within

TABLE 5

Per cent calcium assimilation of cows on timothy hay rations

$$\text{Per cent Ca assimilation equals } \frac{\text{Ca in milk plus Ca in calves minus Ca lost from bones}}{\text{Ca in food}} \times 100$$

COW	BREED	TIME ON RATIONS	DRY PERIOD	AVERAGE DAILY FEED CONSUMED			CALCIUM INTAKE FOR PERIOD				CALCIUM OUTGO FOR PERIOD			ASSIMILATION WHEN LOSS OF BODY Ca IS:			MILK YIELD		PERIOD OF LACTATION
				Hay	Grain	Silage	In hay	In grain	In silage	Total	In milk	In calves	Total	None	15 per cent	25 per cent			
		mos.	*mos.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*					*kgm.*	*months*
429	P. B. Jersey	30	6½	3.60	6.39	6.38	9.575	9.270	5.718	24.563	10.821	0.61	11.431	46.5	42.5	39.8	(1)	2,666	Last 9 of 12½
																	(2)	4,826	Entire 13
																	(3)	768	First 1½
																	Total	8,260	
54	Grade Guernsey	27	5	3.47	6.07	6.49	8.162	7.937	5.235	21.334	5.852	0.36	6.212	29.1	22.8	18.6	(1)	1,702	Last 9 of 13
																	(2)	4,149	Entire 13
																	Total	5,851	
456	P. B. Jersey	32	5½	3.33	4.90	5.11	9.204	7.575	4.884	21.663	6.454	0.61	7.064	32.6	28.0	25.0	(1)	1,090	Last 9 of 12½
																	(2)	2,309	Entire 13
																	(3)	1,211	First 4½
																	Total	4,610	
450	P. B. Jersey	14	3½	3.93	4.71	0	4.760	3.190	0	7.950	2.423	0.21	2.633	33.1	26.1*	21.4†	(1)	1,731	Entire 10½

* Seven and one-half per cent loss of body Ca.
† Twelve and one-half per cent loss of body Ca.
} Since time of No. 450 on ration was only approximately one-half that of other cows; this assumes a linear relation between loss of body calcium and time.

the feeding period, the portion of the lactation, the milk yield for that portion, and the total milk yield for the whole period. Again it is to be noted that Cow 429 secreted much more milk than the other cows on the rations for similar periods of time.

The per cent calcium assimilation was calculated in accordance with the equation at the top of the table. The calcium content of the hay fed was determined. The calcium content of the grain No. 55 fed was not determined. The figure used, 0.159 per cent Ca, is the average of maximum and minimum values of the analyses of two separate lots of Grain 55 fed during a balance experiment carried out at this station. The maximum figure was 0.162 and the minimum 0.156 (1, p. 846). The percentage of calcium in the corn silage fed was not determined. The figure used, 0.0982 per cent Ca, is the average of the maximum and minimum values of analyses of corn silage fed during a balance experiment carried out in this laboratory. The maximum value was 0.1037 and the minimum 0.0926 (3, p. 495).

Henry and Morrison (5) give the calcium content of timothy hay as 2.5 parts CaO per thousand. This would be equivalent to 0.18 per cent Ca, while the average Ca content of the hay fed to our cows ranged from 0.284 to 0.291 per cent for the different cows. The figures given by Henry and Morrison for the calcium content of the constituents of our grain No. 55, would make the calcium content of this mixture 0.172 per cent, which is somewhat higher than our figure, though not nearly enough to compensate for the difference in the timothy figures. No figures are given by Henry and Morrison for the calcium content of corn silage, but this constituent supplied only a rather small proportion of the total calcium content of our rations. From the considerations which have just been given it does not seem likely that the calcium content of our rations has been underestimated.

The calcium content of the milk of No. 429 was 0.131 per cent (February, 1925); that of No. 54, 0.100 per cent, this figure being an average over 29 periods during two balance experiments carried out on this animal (1). The calcium contents of the milks of Nos. 456 and 450 were not determined. The figure used was 0.140 per cent Ca. This is an average of the values for the

calcium content of the milk of Cow 412, 0.142 per cent Ca, that of No. 441, 0.146 per cent Ca (averages of 7 and 6 periods, respectively, during a balance experiment on these animals (6)), and that of Cow 429, 0.131 per cent Ca. All five of these cows are purebred Jerseys of the Beltsville herd.

The calcium content of a calf at birth has been taken as 1.28 per cent. This figure is the average of the values (1.169, 1.261, 1.258, 1.437, 1.273) for five Jersey calves analyzed at the Missouri Agricultural Experiment Station (7). This average value has been used in computing the calcium content not only of the calves of the three Jersey cows, Nos. 429, 456, and 450, but also that of the calves of No. 54, a grade Guernsey.

The calcium content of the body of a mature cow is approximately 1.5 per cent. This figure was calculated by utilizing, with certain approximations, the values of the Ca content of steers at the ages of forty, forty-four, and forty-eight months, slaughtered and analyzed at the Missouri Agricultural Experiment Station (7). This value (1.5 per cent) is practically identical with that calculated from figures given by Hart (8). From data of the Rothamsted Experiment Station, Hart estimated that there were 24.2 pounds of CaO in the body of a Holstein cow weighing 1150 pounds.

In table 5 are given three values for the percentage calcium assimilation, computed by:

1. Assuming no loss of body calcium during the feeding period.
2. Assuming a loss of 15 per cent of body calcium. This is approximately the loss indicated by the decrease in the weight of the bones of Cow 429 as presented in table 4.
3. Assuming a loss of 25 per cent of body calcium. This is undoubtedly a liberal allowance and, other things being equal, the assimilations calculated on this basis are very probably to be regarded as minimum values.

The writers realize keenly the many sources of possible error in the results which have been given. But, in considering these results, and the possible errors to which they are subject, it must be remembered that one of the main objects of investigating calcium metabolism is to determine what proportion of her calcium

intake a cow can utilize for the production of milk and calves on the average, when she is kept under ordinary practical conditions for a long period year in and year out. This question has been investigated in the past chiefly by means of balance experiments, and it must be pointed out that neither the nature of such experiments nor the results that have been obtained from them give any warrant for supposing that they furnish a flawless method for answering the question under consideration. In order to apply their results to answering this question, it must be assumed that results obtained in short periods during which the animals cannot be kept under normal conditions are representative; the justification for this assumption will depend largely on how far the results of different balance experiments agree with each other.

Unfortunately there is no satisfactory agreement among the results of balance experiments. Not only do groups of results obtained by different investigators disagree considerably with one another, but it often happens that different animals studied by the same investigators under apparently identical conditions of feeding and treatment show wide differences in their calcium assimilation (1). It seems justifiable, therefore, to regard the results of our experiments as a valuable check on the results of balance experiments.

The values for calcium assimilation in table 5, calculated on the basis of 15 per cent loss of body calcium, are somewhat higher and those calculated on the basis of no loss of body calcium are considerably higher than those which have been obtained in most[3] balance experiments (about 20 per cent) (1). The difference between individual animals is strikingly evident. Cow 429 assimilated almost 40 per cent of her food calcium, even allowing for a loss of 25 per cent of her calcium stores. It seems, therefore, that good cows may be capable of assimilating a relatively large proportion of their dietary calcium under these conditions.

The basis upon which the figures for 15 per cent loss of boyd calcium have been calculated was, as stated previously, the lower

[3] Ellenberger of the Vermont Station has recently reported (9) percentages of assimilation of 50 per cent and over on rations containing timothy hay unsupplemented with minerals.

bone weight-body weight ratios for Cow 429. Obviously, this ratio is greatly influenced by the degree of fatness or fleshiness of the animal. No estimates of the relative amounts of fat or of flesh of these cows were obtained. But even if the ratios were higher for No. 429 than for the two alfalfa cows, or if the ratios were the same for all three and the theory of the disappearance of the entire bone material upon depletion of the minerals is untenable, this only further emphasizes the fact that the values for calcium assimilation are higher than those obtained in most balance experiments, since then the only determining factor would be the change in composition of the bone. This was found to be little if at all altered by long periods on timothy hay and hence the values of percentage calcium assimilation calculated by assuming no loss of body calcium would be the only basis for comparison with figures from balance experiments.

With regard to the content of mineral matter in bone, age obviously plays a rôle and it might be contended that the bones of Cow 429 (nine years of age) would, if she had been on a high calcium diet (alfalfa hay), have shown a larger mineral content than those of No. 90 (five and three-quarter years of age). However, Cow No. 17 (sixteen years old), a considerably older animal, had a mineral content greater than No. 90's by only about 12 per cent, calculated on the dry basis. It is scarcely to be expected that there would be anywhere nearly as large a difference between Cow No. 429 (aged nine years) and Cow No. 90 (aged five and three-quarter years). A similar conclusion may be reached with respect to the possible effect of the stages of lactation at which the deaths of the animals occurred, or of pregnancy, on the mineral content of the bones. The bones of Cow No. 17, the alfalfa-fed animal, were obtained after she had been dry for from four to five months. Their mineral content was only about 8% greater than those of Cow No. 429, the timothy-fed animal, whose bones were obtained at the end of one and one-half months of lactation. The only one of the three cows which could have been pregnant was Cow No. 90; she was bred just one month before slaughter.

That the determinations of the chemical elements contained in

bone were accurate is to be inferred from the fact that the Ca:P ratios in the bones of all the animals used in this investigation were close to the normal ratio. From data given by Hammarsten (10) the ratio of Ca:P by weight in bone is about 2.15. According to the figures given in table 1 (dry basis), this ratio did not in any case rise as high as 2.23 or fall as low as 2.14 in the bones of the animals studied.

The results agree with those of previous investigations in showing that the Ca:P ratio in the bones of animals cannot be changed by feeding them a ration low in calcium. Curiously enough the proportion of calcium to phosphorus was higher on the average in the case of Cow No. 429, which had been on the low calcium ration, than in that of either of the other two cows. In the bones of Cow No. 429 the Ca:P ratio was about 2.20, while in those of Cow No. 90 and Cow No. 17, it was only 2.16 and 2.17, respectively.

SUMMARY

Four cows were maintained under herd conditions on a ration of timothy hay, a grain mixture, and corn silage for periods of fourteen to thirty-two months.

Analyses were carried out on the bones of one of these cows (thirty months on timothy) and on those of two other animals which had been on alfalfa hay for some time before slaughter. Calculations have been made of the percentages of calcium assimilated by the four cows on timothy hay.

1. The results indicate that the relative amounts of ash, calcium, phosphorus, nitrogen, and organic matter in cows' bones are little if at all altered by long periods on rations low in calcium, even when a considerable amount of milk is given in such periods.

2. The results suggest that the treatment above described reduces by from 10 to 20 per cent the total weight of the bones, and, therefore, the amounts of calcium and phosphorus in the body.

3. The results indicate that, even on a mediocre roughage, the calcium assimilation tends to be somewhat higher in long periods under natural conditions than it has been in most balance experi-

ments, and that, in the case of good cows, it may be much higher than it has been in most balance experiments.

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PROPERTIES OF SOME ACID CASEINS*

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The claim has been made that imported casein is superior to the domestic product and is, therefore, preferred by paper-coaters, the principal users of casein. In order to obtain data on the properties of domestic casein, a number of samples were analyzed and tested within a few days after their receipt from the manufacturers. Data on the first ten of the samples received are presented here. In a majority of the samples the dry casein had evidently been placed in the mailing cases as it came from the trays. The samples were all ground to a uniform size so that practically all of the particles would pass through a 20-mesh sieve.

Appearance. The appearance of the samples after grinding to a uniform size was much the same except for a small variation in the amount of dirt and burned particles. The color was good. Particle size affects color. Differences in this property may best be determined by examining solutions of the samples.

There was no indication that any of the samples had deteriorated because of long storage.

Fat. The method described by Sutermeister (1) for the estimation of fat in casein was used in these tests. This method is a modification of the Babcock method and is much more rapid than procedures which include the use of organic solvents. The condition of the fat at the end of the tests indicated that the concentration and amount of sulphuric acid used was sufficient to dissolve the casein without charring the fat.

Moisture. Approximately 1.0 gram was weighed into a small flat platinum dish and dried in a vacuum oven for five hours at 98.0°C. The weight of the dry casein was obtained and the sample was then used for ash determinations.

Ash. If the sample is low in ash the addition of calcium in the

* Presented at the Annual Meeting of the American Dairy Science Association, Ames, Iowa, June, 1930. Received for publication January 13, 1931.

form of calcium acetate is necessary to prevent volatilization of organic phosphorus during ashing, as shown by Shaw (2). Therefore, in order to prevent any possible loss of phosphorus, 2.0 cc. of approximately M/10 calcium acetate solution (0.0116 gram CaO) was added to each sample before charring it over a micro-burner. Care was taken to prevent the casein from bursting into flame. One cubic centimeter of C. P. concentrated nitric acid was added to each sample. The samples were gently heated before placing them in the muffle. It is difficult to get a white ash without the action of the acid.

Strength. The method used for determining the strength of the casein was essentially the same as the method for strength determinations described by Sutermeister (3). Two or more grams of borax were used as the solvent. The solution was tested in each case with phenolphthalein to be sure that it was alkaline. The method is an empirical one. The numbers 6 to 12 denote the strength of the casein and refer to the grams of casein in each coating preparation. The greater the adhesiveness of the casein the smaller is the number denoting its strength.

A standard paper was used for all the tests. It was a rosin-sized, 100-pound machine-finish book paper obtained from the Government Printing Office.

Sufficient china clay was obtained to last through the determinations so that any variation due to a difference in the clay was eliminated.

Viscosity. A great deal of work has been done by a number of investigators on the viscosity of casein solutions. The methods employed have involved the principle of time of efflux from a standard orifice, or else the torsion of a wire caused by the resistance of the casein solution to the rotation of a plunger. Some observers claim to be able to judge the viscosity or fluidity of casein solutions by simple observation, but it is impossible to do this with any degree of certainty because an opaque creamy solution may look viscous and have a low viscosity expressed in absolute units, whereas, a transparent watery looking solution will often have a comparatively high viscosity.

The great variety of methods employed to determine the viscos-

ity of casein solutions makes it impossible to compare the work of one experimenter with that of another. Sutermeister (2) states, "No one has yet made a study to find out which casein solutions, if any, are *viscous* and whether the more important solutions are to be regarded as *plastic* or perhaps of a still more complicated flow character requiring three or more parameters for its description."

There are many methods of determining the viscosity or consistency of a fluid or mushy material. One of these is the burette consistometer. The fundamental principle of its use is to make a series of observations at different rates of flow by permitting the hydrostatic head to vary. The burette consistometer requires no external source of pressure and serves as a convenient means for obtaining flow-pressure data on materials that are not too stiff. This instrument has been used by Cook (4) and Auerbach (5). Herschel and Bulkley (6) describe Auerbach's apparatus and also report a method of calibrating the burette consistometer and the degree of concordance attainable.

The apparatus and methods used in this work for making determinations of the consistency of the casein solutions were essentially those described by Herschel and Bulkley. The lower end of a 50.0 cc. burette was cut off just above the beginning of the constriction near the stop-cock and suspended in a water bath as shown in figure 2 of Herschel and Bulkley's article. It was then calibrated by means of a cathetometer at each 5.0 cc. mark. The capillary tube was inserted in the lower end of the burette and held in place by a rubber stopper in such a manner that the upper end was always even with the 40.0 cc. graduation on the burette. This prevented cooling or warming of the liquid in the capillary since most of the capillary tube was surrounded by the material being measured. It was decided to make readings at three different heads and to take the time required for the liquid to flow from the 0.0 to 5.0, 10.0 to 15.0, and 20.0 to 25.0 cc. graduations. The average heads were calculated by the formula

$$h = \frac{h_1 - h_2}{\log_e\left(\frac{h_1}{h_2}\right)}$$

where h_1 is the initial and h_2 is the final head. The average heads were 56.31, 45.41 and 34.49 cm. respectively. The length of the capillary was 15.6 cm. and the diameter 0.102 cm. It took from about one to two and one-half minutes for the 5.0 cc. portions to flow from the capillary.

The equation used by Bateman and Sharp (7) was employed for determining the consistency of the casein solutions. In this equation, ξ, the consistency of a plastic substance, corresponds to N, the viscosity of a liquid.

$$\xi = \frac{\pi r^4 g(P - p)t}{8\, lv}$$

where r is the radius of the capillary, l its length, v the volume flowing through the capillary in time t, P the total pressure in grams per square centimeter producing the flow, p the yield value pressure and g the gravitational constant. The only difference between the fundamental equation for viscous flow and that for plastic flow is the substitution of $(P\text{-}p)$ in the latter for P in the former. If p equals zero the substance is viscous; if p has a value greater than zero the substance under investigation is plastic.

It was decided, for reasons which will be described later, to use a 9.0 per cent casein solution with sodium hydroxide as the solvent. The average specific gravity of a large number of the solutions was found to be 1.02, the variation between samples being slight. The value of P for the greatest head, therefore, becomes 56.31 grams per square centimeter (the average head between the 0.0 and 5.0 cc. graduations) times 1.02.

Measurements were made at 30.0°C.

It was found that the value for p at the different heads was zero, so that the values in the formula for the greatest head became

$$\xi = \frac{(3.1416)\ (0.051)^4\ (981.0)\ (56.31 \times 1.02)\ (t)}{(8)\ (15.6)\ (5.0)}$$

ξ for any definite head may therefore be expressed as $\xi = K \times t$ so that the value for ξ at the different heads was $0.00192 \times t$,

0.00155 $\times$ t and 0.001177 $\times$ t. It was necessary only to multiply any one of these constants, depending on the head used, by the time in seconds required for 5.0 cc. of the casein solution to pass through the capillary to obtain the viscosity of each solution in poises. Since the solutions were viscous and not plastic, the determinations of the viscosity in poises at the three heads was a check on the accuracy of the determination. It is important to check by this method since small lumps in the solution in the capillary decrease the rate of flow and increase the value obtained for the viscosity.

Zoller (8) employing sodium caseinate solutions at pH 9.0, found that a 9.0 to 10.0 per cent caseinate solution is in the region of maximum sensitiveness to differences in reaction and to other factors such as salts, or ion effects. This experiment, as described by Zoller, has been repeated with substantially the same results. It was decided, therefore, to use 9.0 per cent solutions for the viscosity determinations and to adjust the reaction to a pH of approximately 9.1 to 9.2. Zoller also showed that a maximum viscosity occurs at about pH 9.10 to 9.25. Attempts by the authors to check these results were not successful. The viscosity somewhat above pH 9.1 to 9.2 increased, but not as rapidly as at less alkaline reactions.

It is known that casein solutions change in viscosity with time. Accordingly, a standard procedure was adopted for dissolving the casein in N/1 NaOH and making up the solution to approximately the concentration and reaction desired, allowing it to stand in the 10.0°C. cooler over night, adjusting the reaction and concentration to the exact point the next morning and making the measurements in the afternoon. The concentration of the solutions was based on the wet casein and the adjustment to the desired pH value was made in the beaker containing all of the solution. The antimony electrode was used. It was standardized with buffer solutions of known pH value and the pH of the sodium caseinate solutions as determined with the antimony electrode was found to check with the value given by the Bailey electrode method.

The reaction of the samples was determined by three methods:

1. Free acid. The method used for determining this property is empirical and was chosen because of its use in the casein industry. Ten grams were thoroughly mixed with 100 cc. of distilled water and the mixtures allowed to stand for one hour with occasional stirring. Fifty cubic centimeters of the super-

TABLE 1

Properties of some acid caseins

SAMPLE NUMBER	WATER	ASH	FAT	FREE ACID	REACTION	ALKALI TO DISSOLVE TO pH 9.15 ± 0.05	VISCOSITY	STRENGTH	REMARKS
	per cent	*per cent*	*per cent*	*cc. N/10 NaOH*	*pH*	*cc. N/1 NaOH*	*centi-poises*		
1	5.91	1.93	0.27	1.0	4.74	8.0	14.09	7	Coating mixture and solubility excellent
2	7.22	2.68	0.00	5.6	4.60	8.5	7.51	7	Coating mixture and solubility good
3	7.86	3.89	0.18	6.4	5.00	7.0	14.73	9	Coating mixture lumpy. Solution thick
4	8.03	3.76	0.18	6.2	5.03	7.0	12.22	11	Coating mixture slightly lumpy. Solution thick
5	8.35	3.90	2.70	5.0	5.20	6.0	13.88	9	Coating mixture slightly lumpy. Slowly soluble
6	7.48	5.11	0.45	7.8	5.14	6.2	8.57	12	Coating mixture and solution thick
7	6.23	3.63	0.18	13.0	4.32	10.2	14.36	11	Coating mixture smooth. Solubility fair
8	6.58	3.05	1.08	14.2	4.25	10.5	11.36	12	Coating mixture thick. Slowly soluble
9	6.30	3.39	0.09	15.2	4.35	9.6	11.66	11	Coating mixture and solubility poor
10	6.49	4.34	0.00	16.2	4.46	10.2	17.40	12	Coating mixture poor. Very insoluble

natant liquid were then titrated with N/10 NaOH with phenolphthalein as the indicator. The value given for free acid is the number of cubic centimeters of alkali required to neutralize the acid in the 50.0 cc. of supernatant liquid.

2. pH of casein. Two grams were well mixed with 2.0 cc. of distilled water and the pH determined by the quinhydrone

method in the same manner as was employed by Watson (9) for obtaining the pH of cheese.

3. The reasons for adjusting the reaction of the solutions employed for making viscosity determinations to pH of about 9.15 have been given. The cubic centimeters of N/1 NaOH required to do this depends upon the free acid in the casein and chiefly upon the pH at which the casein was put to press.

Solubility. The samples have been listed in the order of their solubilities as determined by inspection when making up the solutions for the viscosity and strength measurements.

The solutions of numbers 6, 7, and 10, which were made up for viscosity determinations, were milky in appearance and considerable difficulty was experienced in dissolving many fine white specks which looked like particles of insoluble casein.

Data on the samples of domestic casein obtained by the methods described are presented in table 1.

DISCUSSION

No definite standards have been established for the grading of casein. The domestic casein market would be better than it is if the manufacturers received a price based more on quality, as is the case in the marketing of other dairy products such as butter and dry skim milk.

The quality of casein is important. The manufacturer should, therefore, be concerned more about carrying out a process which will result in a product of good quality rather than in one which may give the largest yield.

It is not possible to judge the quality of casein from its general appearance. Dirt indicates lack of care in keeping equipment in good condition and may mean poor quality because of faulty manufacturing methods.

The properties of casein which is satisfactory for paper coating are not generally established. Strength is an important factor. If this were the only consideration, casein having a strength of 6 should sell for twice as much as casein having a strength of 12. A strong casein usually possesses other desirable properties.

The viscosity of the casein solution influences the manner in

which the coating preparation will spread. Several of the values given in the table are low in this respect. An average figure for this property (based on unpublished data) for solutions tested as described above, is about 14.0 centipoises.

Much importance is attached to the borax solubility test as a criterion of quality. This test not only indicates the rate of solution, but also shows the presence and number of insoluble particles. It differentiates between caseins made at high and low temperatures. Cooked curd caseins tend to imbibe water and form a jelly.

The reaction of casein is influenced chiefly by the pH at which it was put to press and also by the amount of free acid present. If the whey is drained from the curd at the right degree of acidity and if the curd is washed effectively, the dry casein will have a pH of about 4.6, a low free acid value and also a low ash content.

The data in table 1, relating to free acid, show a wide variation. This acid must be neutralized before a coating preparation can be used and during the neutralizing process salts are formed which cause trouble, especially when satin white is used. If the casein is made so as to contain practically no free acid, less alkali will be needed and foaming is less liable to result.

The data in the table show properties of ten samples of commercial domestic casein. Only samples 1 and 2 can be classed as satisfactory. These samples are not presented as being representative of domestic casein, but it is not a good recommendation for the domestic product that so many of the samples obtained were so low in quality.

Most of the factors that affect the quality of one type of acid-precipitated casein affect all types. First of all sweet, clean, fat-free skim milk should be used. The keeping quality and strength of casein is decreased by the presence of fat and of course the return for the fat is much less than it would be if it were sold as cream or butter.

The precipitating temperature is important, especially where hydrochloric acid is used as the precipitating agent. When hydrochloric acid casein is made a temperature of 94 to 96°F. is necessary for best results. Below 94°, soft, fine curd forms that

is hard to drain and wash, and which results in excessive mechanical loss in processing.

It is evident from analyses made by the Bureau of Dairy Industry of commercial samples of casein that the reaction of the whey at the time when the vat is dipped is not properly controlled. This should be at pH 4.1 or a titratable acidity of approximately 0.50 per cent measured as lactic acid with phenolphthalein as the indicator when the hydrochloric and sulphuric acid types are being made. The acid should be added slowly and the milk stirred constantly. Too fast stirring gives too fine a curd, and too slow stirring a lumpy curd. An excess of acid causes the formation of a gelatinous layer over the particles. This tends to make them stick together and injures their texture. Insufficient acid gives a milky, soft curd. In either case it is difficult to drain and wash the curd properly.

Casein occurs in milk in combination with calcium. The development of acidity (due either to fermentation or the addition of an acid) causes this calcium to be released and the casein to be precipitated. The acidity of the contents of the vat should be carried beyond the point at which, theoretically, the casein would be free of calcium. This is done to hasten the release of the calcium and to aid in the formation of a curd which will have a desirable texture.

The concentration of the acid used is usually too great. If sulphuric acid is diluted approximately 1:30, it will be found that a granular curd results from which the ash and acid can be much more easily washed, than if the acid is diluted 1:4 or 1:10.

The curd should be thoroughly drained and washed. The longer it is allowed to remain in contact with the whey the harder it is to wash it free of impurities. The curd still contains approximately 50 per cent moisture after it has been well pressed and is, therefore, readily subject to deterioration changes. The curd should therefore be dried as soon as possible after it is taken from the press.

So long as considerable water is present in the ground curd, it will not burn easily. If the curd is heated too quickly in the drier it will case harden. The first and last stages of the drying

operation, then, should be watched more closely than the intermediate stage. So long as the final product contains less than 8 per cent moisture, it will not deteriorate quickly.

Insufficient attention has been given to the manufacture of casein. It should be carried out as a chemical process. Lack of realization of the importance of the manufacturing steps mentioned above is largely responsible for the quality of our domestic casein. It is not difficult to make high-grade casein, and when this is done the users will show their appreciation by paying a premium for it.

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METHOD FOR HYDROGEN ION DETERMINATION OF BUTTER*

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Acidity has long been recognized as a factor of primary importance in influencing the keeping quality of butter. One phase of the problem of manufacturing salted butter of good keeping quality,—the prevention of the development of fishy and related off-flavors—is intimately related to the acidity of the cream at churning time and consequently the acidity of the butter. Unexplainable variations from the normal and expected results in isolated instances in storage of butter have cast some suspicion on the adequacy of the determination of acidity by titration methods. The true acidity as determined by hydrogen ion concentration may conceivably furnish a more reliable measure of the ability of butter to withstand at least this one type of deterioration.

The solution of this problem by means of hydrogen ion control of the cream at time of churning is a question that presents itself for future consideration. The work reported herein has to do with the hydrogen ion concentration of butter and suggests the possibility of the development of a test indicating the suitability of butter for cold storage. Preliminary studies and observations by the authors confirmed in a general way their earlier belief that there is a correlation between the pH of butter and its keeping quality. The results of our studies on the relation of pH value to keeping quality will appear in a later paper. The present paper is confined to a presentation of methods for determining the hydrogen ion concentration of butter that have been evolved from considerable experimentation. The establishment of these methods is the result of a study of the peculiarities of butter and the modification and precautions necessary to secure comparable and dependable results.

* Received for publication January 23, 1931.

DISCUSSION OF LITERATURE

Aside from the work of Parfitt (1), of Haglund, Platon, Waller and Soderberg (2), and of Lundstedt (3), the literature shows no reference to hydrogen ion determinations of butter and the relation of the hydrogen ion concentration to the keeping quality of butter.

Parfitt compared the pH value of butter before and after storage. His method consisted of melting the butter at 45°C., making a 1 to 10 dilution and comparing 1 cc. of this dilution in LaMotte (4) little dishes with LaMotte indicators. We have tried out carefully and extensively the method used by Parfitt and also the colorimetric method recommended by Sharp and McInerney (5) for milk. We have used dilutions of 1 to 1, 1 to 5, 1 to 10, 1 to 20, and 1 to 50 of both the serum of the butter and of the butter itself, employing the following indicators: brom cresol purple, brom thymol blue, methyl red, phenol red, chlor phenol red, and brom cresol green. As will be indicated later, neither the butter nor the butter serum can be diluted with water without affecting the pH reading. Whether it is possible to use a factor for butter, as is suggested by Sharp and McInerney for milk, we are not as yet prepared to say. Our results show marked irregularities caused by such dilutions of different types of butter. Likewise, the choice and behavior of indicators is accompanied by many inconsistencies in results. Our findings in regard to the colorimetric method are based on careful comparisons of colorimetric readings with electrometric determinations using both the quinhydrone and the hydrogen electrode.

We recognize the distinct advantages of the colorimetric method from the standpoint of simplicity of apparatus and procedure, and of economy of apparatus and butter samples. However, our work so far fails to show that this method is sufficiently reliable to justify its use for the pH determination of butter. We hope to show conclusively in a later paper if the colorimetric method can be made applicable to butter.

Haglund, Platon, Waller, and Soderberg studied the relation of the hydrogen ion concentration to the keeping quality of butter. Their pH determinations were made by the quinhydrone method, but the details of their procedure are lacking.

Lundstedt compared the hydrogen electrode with the quinhydrone electrode on various dairy products. This work came to our attention after preparing this paper for publication. His results show close agreement between the quinhydrone and the hydrogen electrode methods. Lundstedt found no difference between the pH of the undiluted and diluted portions of buttermilk, butter culture, and cream, and concluded that such slight dilutions in no way affected the measured potentials. This is somewhat at variance with our results and those of other investigators. As the result of his comparisons of the pH of butter between the quinhydrone and the hydrogen electrodes he concluded that no special salt or protein effects were apparent.

FACTORS INVOLVED IN THE PREPARATION OF THE BUTTER SAMPLE

Various procedures are in use for the preparation of products for pH determinations. Wherever possible, the use of the material in its original form is given preference. The peculiar make-up of butter, however, does not permit the determination of the hydrogen ion concentration on the whole butter in unmodified form as is the case with other dairy products, such as milk, cream, cheese, ice cream, condensed milk, etc. The butter fat comprising over 80 per cent of the composition of butter is not an electrolyte and refuses to establish connection between electrodes.

The preparation of the butter, therefore, becomes of fundamental importance. The method of preparation must be such as to avoid altering the pH of the butter, as far as possible, and the sample must be of adequate size and representative of the material to be examined.

In dairy products changes in reaction occur so quickly that the element of time may become very important. This is a factor with butter as it must be melted. The electrometric determination of pH on the butter direct and without dilution or removal of the fat is not feasible. This was conclusively established experimentally. No readings were obtained on butter unless the serum was allowed to separate out. These findings were further confirmed by using the method described by Watson (6) as satis-

factory for cheese. In this method a small amount of the cheese and the required amount of quinhydrone powder are mixed together to form a paste which is placed in a tube containing the metal electrode. The same method tried with butter gave no readings at all, suggesting that in the butter-quinhydrone paste the fat constitutes the continuous phase. However, if the butter fat is allowed to separate, contact through the serum portion at the bottom is established and a reading is obtained. This method is not satisfactory, however, because of the fouling of the electrode with fat, and because of the difficulty of accomplishing a suitable mixture of the quinhydrone powder with the serum. In the presence of so much fat, the bulk of the quinhydrone powder remains in the fat and is lost to the serum where it is needed for the correct pH determination.

Because the fat is not an electrolyte its removal probably in no way influences the actual pH value of the butter. The fat may be regarded as an inert foreign substance, the presence or absence of which is of no consequence in the results of pH determinations of butter. In other words, the pH of butter is, in fact, the pH of the butter serum.

In arriving at these deductions we are cognizant of a possible fatty acid liberation resulting from partial decomposition of the fat. However, the water-soluble fatty acids, which would appear to be the only group of fatty acids capable of affecting the pH readings, should become a part of the serum when the butter is melted and, therefore, enter into the reaction of the butter serum.

APPARATUS FOR THE ELECTROMETRIC pH DETERMINATION

The apparatus used in the present procedure consists essentially of two parts, the voltage recording apparatus and the electrode assemblage. A Leeds and Northrup (7), Type K, potentiometer, a sensitive light ray galvanometer, and a two-volt storage battery with a Weston standard cell arranged for easy standardization of the storage battery voltage, constitutes the equipment for recording the minute voltages obtained. The standard cell was calibrated by the Bureau of Standards and has not suffered any noticeable change in voltage over a period of five years.

The electrode assemblage consists of three parts, the two electrodes and the vessel containing the liquid under examination. One of the electrodes is the calomel electrode, and the other is the hydrogen electrode or other electrode used in its place.

The exact type of calomel electrode is not so essential. It should be connected with a supply reservoir so that frequent flushing out with fresh KCl solution is possible. Only the very purest chemicals should be used in its preparation. Our experience has demonstrated that errors as great as 0.3 to 0.4 pH may result from the use of chemicals and electrodes that are not of extreme purity. About 1 ounce electrolytic mercurous chloride and about 8 ounces of redistilled mercury, such as prepared by the Eppley Laboratories, Newport, R. I., should be procured. With extreme care in its preparation the calomel electrode will last almost indefinitely and need never be changed.

The saturated KCl calomel electrode appears to be the most convenient although we have also used the N/10 KCl electrode. The only disadvantage in the use of the saturated KCl solution is its tendency to creep out at rubber connections and up over the edge of the salt bridge container. Such creeping may be readily prevented by applying a thin film of vaseline at the rubber connections and around the upper part of the salt bridge container immediately above the surface of the KCl solution.

To prevent undue crystallization of the KCl in the calomel cell and the KCl reservoir, the temperature of the surrounding air should not be permitted to drop much below about 20°C. To facilitate a rough control of the air temperature the authors placed their hydrogen ion apparatus in a cabinet of suitable size, and have been able to maintain the temperature of this cabinet between about 20° and 27°C. by the use of two nichrome "Sun Bowl" heater elements in series.

A small beaker serves admirably as the vessel for the solution to be tested. Some provision should be made to maintain the temperature of the solution under test precisely at the desired temperature, as even minor fluctuations in temperature markedly affect the voltage reading obtained. The authors provided a simple thermostatically controlled water bath in which the beaker containing the solution to be tested was immersed. A U-tube

filled with 1.5 per cent agar in saturated KCl solution provided connection between the salt bridge and the test solution beaker. These U-tubes must be kept immersed in saturated KCl solution when not in use. They can be used repeatedly without any noticeable change in conductivity. The darkening effect of quinhydrone mixtures on the end of the immersed U-tube appears not to affect their accuracy.

Several different types of electrodes were used. These were the usual hydrogen electrodes, the quinhydrone electrode, and the antimony electrode. Of the various hydrogen electrodes used the simpler Hildebrand electrode with a No. 22 platinum wire protruding about $\frac{3}{16}$ inch from the glass tip appeared to be the most suitable. Difficulty in readily and thoroughly cleaning the plate type of electrode appeared to be a marked disadvantage here. To secure the best deposit of platinum black the authors used about $\frac{1}{2}$ ounce of a solution containing 3 grams of platinic chloride and 0.025 gram lead acetate per 100 cc. water. Two electrodes are preferably plated at the same time by connecting to the terminals of a two- or three-volt battery and reversing the current every fifteen to thirty seconds. Deposition for one to two minutes elapsed time usually gave the best results. The electrode must be thoroughly rinsed and kept in distilled water when not in use. Ordinary tank hydrogen gas was found sufficiently pure, no advantage being observed in the use of the customary gas washing chain. When in use the hydrogen gas should be passed through the electrode at a rate of about two bubbles per second.

Usually about twenty to thirty-five minutes was required for equilibrium to be reached in the case of the dairy products examined in the present experiment. The electrodes were periodically checked by the use of buffer solutions such as recommended by Clark (8), and the acetic acid sodium hydroxide buffer obtainable from Leeds and Northrup (7). The pH value for the hydrogen electrode was calculated by the use of the following formula, at 25°C.

$$\text{pH} = \frac{\text{voltage obtained} - 0.2458}{0.0591}$$

After considerable experimentation the quinhydrone method of Biilmann (9) with a gold spiral electrode was found to be the most suitable. A reversing switch, however, was required in the circuit since all voltages for a pH below about 7.7 are of an opposite sign as compared with the usual hydrogen electrode method. After each use the gold spiral electrode must be cleaned by rinsing in hot distilled water and from time to time in a 1 to 1 solution of ethyl alcohol and ethyl ether. The electrode must then be kept immersed in distilled water when not in use. The following formula was used for converting voltages obtained by the quinhydrone electrode into hydrogen ion readings, at 25°C.

$$\text{pH} = \frac{0.6990 - \text{voltage obtained} - 0.2458}{0.0591}$$

As will be shown later, results obtained by the quinhydrone method on butter serum agree very well with the usual hydrogen electrode method. However, with buffer mixtures the quinhydrone method appears to give results a trifle higher, i.e., about 0.05 pH to 0.08 pH above that obtained by the hydrogen electrode.

The antimony electrode has been suggested by some students of hydrogen ion work (10). This electrode does not require hydrogen gas nor other material in the solution, such as quinhydrone. It consists merely of a piece of metallic antimony, about $1\frac{1}{2}$ to 2 inches long and about $\frac{1}{4}$ inch in diameter with a copper wire soldered to it. This electrode is immersed directly in the liquid to be tested and is connected with the calomel cell in the usual way. It was believed that the antimony electrode might be used advantageously in certain phases of pH control, because of its simplicity. It suggests itself as applicable in the factory, as for instance, for pH determinations taken at the cream vat in connection with neutralization and ripening, and also as a rugged electrode for factory pH determinations on butter. For above reasons, we have compared the antimony electrode with the quinhydrone electrode, which latter had been selected for our present experiment. These comparisons, however, are not final and the tables given below are offered merely to show what

results we obtained by the use of this electrode. The formula used for the antimony electrode at 25°C. is:

$$pH = \frac{\text{voltage obtained} - 0.050}{0.054}$$

Table 1 shows that very close agreement is possible between the antimony electrode and the quinhydrone on buffer solutions. On dairy products, however, the agreement observed so far was not

TABLE 1

Comparison of antimony and quinhydrone electrodes on buffer solutions and on milk and butter

MATERIAL TESTED	QUINHYDRONE ELECTRODE	ANTIMONY ELECTRODE
	pH	*pH*
Buffer solution:		
pH = 7.6	7.58	7.61
pH = 7.0	7.02	7.00
pH = 6.6	6.60	6.57
pH = 6.0	6.00	6.01
pH = 8.0	7.96	8.04
Sweet whole milk	6.62	6.85
Sour milk	4.29	4.20
Butter:		
Lot no. 1446	6.68	6.35
Lot no. 575 A	6.35	6.10
Lot no. 575 B	6.53	6.77
Lot no. 575 C	6.88	6.95
Lot no. 556 A	4.71	4.79
Lot no. 556 B	4.99	4.95
Lot no. 556 C	5.27	5.25
Lot no. 863	6.31	6.36

quite so consistent. Incidentally it was noticed that the surface of the antimony tends to darken upon use, and can be brightened by the use of fine emery cloth or steel wool. Whether such discoloration may jeopardize the accuracy of the pH readings is as yet undetermined.

EXPERIMENTAL

The method adopted in the present investigation was to determine the pH of the curd-serum, separated from the melted butter,

by the quinhydrone method at 25°C. Our procedure was as follows:

Preparation of sample. Melt ½ to 1 pound of butter in a 1 quart sealed Mason jar, either by immersing the jar in hot water or by placing in a steam-water oven at a temperature of 75°C. Usually about one hour was required to completely melt 1 pound of butter and secure a satisfactory separation of the curd-serum from the fat. The butter so melted usually reached a temperature of approximately 55°C. No noticeable loss of moisture was observed by this method of melting. Before withdrawing any portion of the curd-serum shake the jar with a rotary motion so as to obtain a uniform mixture and to allow the serum to wash up into the fat. Now let the melted butter settle for a few minutes; then carefully remove as much as possible of the curd-serum with a 25 cc. pipette from below the fat. In the case of a 1 pound sample of butter from 70 to 85 cc. of curd-serum is usually obtained in this way. The first 25 cc. withdrawn is generally fairly clear serum, the remaining portion containing the curd. Mix all portions of curd-serum well for the pH determination.

Determination of pH by the quinhydrone method. Place about 50 mgm. of quinhydrone powder in a 100 cc. beaker, add to this about 1 cc. of the curd-serum and stir to a smooth paste. Then add the remainder of the 25 cc. curd-serum and mix with the paste until thoroughly homogeneous. Adjust the temperature of the mixture to exactly 25°C. and make the pH reading at this temperature, the beaker being immersed in a water bath thermostat.

Comparison of pH values by the quinhydrone and the hydrogen electrode methods. In order to confirm the accuracy of our results with the quinhydrone electrode, numerous check tests on the same samples of butter were made with the hydrogen electrode. The preparation of the samples was identical to that used for the quinhydrone determination except, of course, that no quinhydrone powder was used. This was in addition to comparisons made on standard buffer solutions and on milk and buttermilk on which the literature already contains several references to the close agreement possible between the two meth-

ods. Our purpose for making this comparison on the butter serum itself was to determine whether the quinhydrone powder reacted in any way with the constituents of the serum or interfered with the correctness of pH values of butter.

The pH values shown in table 2 cover determinations on the whole curd-serum of butter. These results show close agreement between the pH values obtained with the two electrodes. Differences were small and in no case exceeded 0.09 pH. Also the variations were in either direction, averaging 0.04 pH. The total average difference in pH values was only 0.01 pH. These comparisons thus serve to confirm the accuracy of the quinhydrone method for pH values within the usual range of hydrogen ion concentrations of butter.

TABLE 2

Comparison of quinhydrone and hydrogen electrodes on butter

SAMPLE OF BUTTER	QUINHYDRONE ELECTRODE	HYDROGEN ELECTRODE	DIFFERENCE	
			Quinhydrone higher	Hydrogen electrode higher
	pH	*pH*	*pH*	*pH*
1	6.13	6.12	+0.01	
2	6.07	6.04	+0.03	
3	6.18	6.13	+0.05	
4	6.41	6.36	+0.05	
5	6.73	6.71	+0.02	
6	5.74	5.65	+0.09	
7	5.54	5.58		−0.04
8	4.61	4.61	00	
9	6.09	6.04	+0.05	
10	5.90	5.94		−0.04
11	6.66	6.60	+0.06	
12	6.58	6.61		−0.03
13	6.73	6.77		−0.04
14	5.02	5.06		−0.04
15	5.48	5.52		−0.04
Average.....	5.99	5.98	0.04	0.04

In considering these results it should be understood that they were not obtained on the butter as such, but upon the curd-serum

of butter from which the fat had been mostly removed. It was impossible either with the Clark shaking electrode or the Hildebrand electrode to secure readings on undiluted melted butter even at temperatures high enough to keep the butter in perfectly melted condition (35°C. or above). However, with the quinhydrone method run at such high temperatures, direct determinations on butter were possible upon allowing the serum to settle sufficiently so as to provide connection between the electrodes. As an example of these experiences the following observations on the pH readings by the quinhydrone method of four variously prepared samples of the same butter are given:

Sample 1

	pH reading corrected to 35°C.
85 grams of butter melted in 100 cc. beaker at 35°C. 200 mgm. of quinhydrone added and mixture well stirred. pH reading on this while stirring continued was	None
Stirring was then discontinued and curd-serum mixture was allowed to settle. In this condition the following reading was obtained	5.86

Sample 2

85 grams of butter melted as above and fat poured off from serum as completely as possible. Quinhydrone powder added to serum remaining in beaker; pH reading was	5.80

Sample 3

130 grams of melted butter centrifuged in a separatory funnel at 35°C. and curd-serum removed. pH reading on curd-serum was	5.85
This method involves the added difficulty of completely reclaiming the curd from the funnel.	

Sample 4

300 grams of butter melted in a sealed Mason jar. Curd-serum pipetted from bottom of jar, as described under "Preparation of sample." pH reading on curd-serum at 25°C. was	5.83

The above examples show that pH readings cannot be made conveniently on the whole butter except when the curd-serum is permitted to separate and in such cases only when run at 35°C. to prevent the fat from solidifying. When the curd-serum alone is used for the sample, instead of the whole butter, the readings can all be made at 25°C. It was to eliminate these various

difficulties of technique encountered when attempting to use samples of the whole butter that we standardized on the curd-serum alone as the most practicable and reliable method for the determination of the pH of butter.

FACTORS INFLUENCING ACCURACY OF METHOD

In the preparation and use of the butter sample for pH determinations, as described in previous paragraphs, certain factors were encountered which required further study. The more important of these are the temperature to which the butter sample

TABLE 3

Effect of temperature used in preparation of sample on pH reading

TREATMENT OF SAMPLE	CHURNING NUMBER		
	1	2	3
	pH	*pH*	*pH*
Butter melted at 75°C.	6.04	7.25	7.32
Buttermilk not heated, fresh	5.95	6.78	6.86
Buttermilk not heated, held at 5°C. for 1 hour		6.75	6.84
Buttermilk not heated, held at 5°C. for 1½ hours		6.73	6.81
Buttermilk not heated, held at 5°C. for 2½ hours	5.90	6.68	6.79
Buttermilk not heated, held at 5°C. for 7 hours	5.85		
Buttermilk heated in water oven at 75°C. for 1 hour		6.53	6.81
Buttermilk heated in water oven at 75°C. for 1½ hours		6.41	6.76
Buttermilk heated in water oven at 75°C. for 2½ hours	5.69	6.42	6.76
Buttermilk heated in water oven at 75°C. for 7 hours	5.63		

is heated for melting and for satisfactory curd-serum separation, the relative effect on pH readings of the curd and serum part of the curd-serum mixture, and the effect of dilution of the sample.

Effect of temperature to which butter is heated. It has been shown by the work of Duncombe (11), Cosmovici (12), Whittier and Benton (13), and Sharp and McInerney (5), that the heating of milk causes a slight increase in the hydrogen ion concentration as shown by a slight drop in the pH reading. These findings are important because of their possible relation to the accuracy of pH determinations on butter, for in order to secure a satisfactory separation of the curd-serum, butter must be heated to temper-

atures considerably in excess of the normal temperature (25°C.) at which the readings are made. In the present experiment, as previously stated, the butter was held at 75°C. for approximately one hour's time and the temperature of the melted butter was usually about 55°C. In order to determine the possible effect of such temperature and time exposure, buttermilk was heated to various temperatures and the pH was determined on the samples

TABLE 4

pH values of butter and buttermilk

SAMPLE NUMBER	BUTTERMILK	BUTTER, I.E. CURD-SERUM OF BUTTER	AVERAGE	
			Buttermilk	Butter
	pH	*pH*	*pH*	*pH*
1472	5.94	6.03		
1473	5.94	6.03		
1478	5.84	6.00		
1479	5.72	5.83	6.02	6.05
308	6.35	6.31		
309	6.37	6.29	Average for churnings in which the pH of the buttermilk was below 6.50	
311	6.34	6.16		
623	4.76	4.83		
328	6.47	6.43		
329	6.40	6.48		
1017	6.09	6.21		
1008	6.66	7.00		
1011	6.73	7.13	6.76	7.11
1023	6.82	7.06	Average for churnings in which the pH of the buttermilk was above 6.50	
1025	6.73	6.88		
946	6.78	7.25		
108	6.86	7.32		

so heated as well as on the butter from the same churning, as shown in table 3.

The results given in table 3 indicate that the heating of buttermilk does depress the pH reading slightly. This is in harmony with the results on milk reported by other investigators. The buttermilk temperature rose to from 52° to 55°C. during the one hour heating period and the reading dropped approximately 0.2 pH. It appears reasonable to assume, therefore, that the same

treatment of the butter sample, in order to obtain the curd-serum separation, may have a similar depressing effect on the pH value of butter.

Additional information on the effect of melting of the butter sample on the pH reading was obtained by melting a sample in running hot water in 15 minutes time. The pH of the curd-serum of this sample was 6.48 while three other samples of the same butter melted in the usual way gave readings of 6.41, 6.38, and 6.39. This would indicate that the standard method of heating for one hour does have a slight depressing effect on the pH.

This slight decrease in pH suggests the possibility of bacterial action with its resultant increase in acidity. However, plate counts on litmus-lactose agar made on samples of both butter and buttermilk before and after heating showed a definite, significant reduction in number of colonies as a result of the heating. Furthermore, plates incubated at 62°C. showed no growth, indicating the absence of thermophiles. A modification of the methylene blue test on the same sample of buttermilk, before and after heating, showed a definitely longer reduction time for the sample after heating, e.g., ten minutes as compared to two hours. These results indicate not only that bacterial growth is not involved but rather point toward a destruction of bacteria during the heating of the sample; hence the decrease in pH must be due to factors other than bacterial action.

Comparisons of the pH of buttermilk and butter from the same churning show a higher pH reading for the butter, notwithstanding any depressing effect of the heating of the sample. This is shown in table 4.

The pH values in table 4 refer in each case to butter and buttermilk from the same churning, and show that the differences in pH vary somewhat with the range of pH. Churnings in which the buttermilk has a relatively high pH yielded butter with a greater difference between butter and buttermilk than churnings in which the pH of the buttermilk was not so high or was low. The eleven churnings showing a buttermilk pH below 6.5 averaged a difference between buttermilk and butter of only 0.03 pH, the buttermilk having a pH of 6.02 and the butter a pH of 6.05.

The six churnings showing a buttermilk pH above 6.5 averaged a difference between butter and buttermilk of 0.35, the buttermilk averaging 6.76 and the butter 7.11 pH.

The figures in table 4 further support the results indicated in table 3, that our method of preparing the butter sample by melting in a water oven at 75°C. has only a slight effect, if any, on the pH value of the butter. In these experiments, the buttermilk sample was not heated while the butter sample was heated in accordance with the method already described. Yet the 17 churnings show an average of 0.14 pH higher for the butter than

TABLE 5

pH values of cream, buttermilk, unwashed butter granules, and finished butter of the same churnings

CHURNING NUMBER	CREAM	BUTTERMILK	UNWASHED BUTTER GRANULES	FINISHED BUTTER
	pH	*pH*	*pH*	*pH*
1008	6.71	6.66	6.96	7.00
1011	6.73	6.73	7.16	7.13
1017	6.15	6.09	6.07	6.21
1023	6.87	6.82	7.10	7.06
1025	6.75	6.73	7.05	6.88
1026*	6.75	6.68	6.88	6.48
Averages.....	6.66	6.62	6.87	6.86

* Omitted from average, 4 gallons of culture worked into 900 pounds of butter with the salt.

for the buttermilk. Assuming that the butter and the buttermilk should have the same pH value, which is not as yet proven, the above would indicate that, if the heating of the butter sample caused any decrease in pH, such decrease must have been slight, as it appears to be more than offset by other factors not as yet determined. An interesting relationship is further shown in table 5, in which the pH values of cream, buttermilk, butter granules before washing and salting, and the finished butter are compared.

The figures in table 5 show very close agreement of pH values between cream and buttermilk. Where there is a difference the

pH of the cream is slightly higher than that of the buttermilk, the maximum difference is 0.07 pH and the average 0.04 pH. Omitting from consideration churn lot 1026 because culture was added to the butter, the finished butter averaged only 0.01 pH lower than the unwashed granules. The unwashed butter granules averaged 0.25 pH higher than the buttermilk, suggesting that there may be an uneven distribution of the factors affecting the pH readings when such sour, neutralized cream is churned. The tendency for the curd-serum to show a higher pH than the buttermilk has masked any slight depressing action of the heating of the sample that might have occurred. The working of culture into the butter lowered the pH of the finished butter 0.4 below that of the unwashed butter granules.

In considering these results attention should be called to the fact that all of these churnings were made from sour, neutralized cream. It is conceivable that the presence of a neutralizer may have some effect on the pH readings in both buttermilk and butter that would be absent in the case of buttermilk and butter made from sweet cream or from ripened cream not neutralized. The effect of neutralization on the relation of pH of buttermilk to pH of butter will be shown in a later paper.

Effect of curd on the pH reading. In preparing the butter sample every effort was made to accomplish as complete a separation of the curd-serum mixture from the fat as possible. Centrifuging, dilution and washing with water, and removal of the curd-serum from the melted butter sample by means of a pipette were compared.

The centrifuging of the melted butter was conducted both in 250 cc. bottles and in 250 cc. separatory funnels. This method proved unsatisfactory because a layer of curd which was difficult to remove packed solidly against the bottom of the flask and against the stopcock of the separatory funnel. Furthermore, below the fat there was a layer of mixed fat and curd which made complete separation of the fat and satisfactory reclaiming of the curd difficult.

In the method by dilution a definite amount of freshly boiled distilled water was added to the melted butter and the sample

shaken to wash out the curd-serum. In other cases water was used to wash the fat after withdrawing the curd-serum. It will be shown later that dilution of the butter serum is not advisable because of its disturbing effect on the accuracy of the pH reading.

Because of the above findings, both centrifuging and dilution were abandoned and the simple method of merely pipetting off the total amount of curd-serum from below the fat layer of the

TABLE 6

pH in aqueous portion and in curdy portion of buttermilk

SAMPLE NUMBER	TEMPERATURE OF SEPARATION	pH IN BUTTERMILK		DIFFERENCE + CURDY PORTION HIGHER − AQUEOUS PORTION HIGHER
		Aqueous portion	Curdy portion	
	°C			
1	63	5.55	5.67	+0.12
2	115	5.59	5.63	+0.04
3	Not heated	5.85	5.81	−0.04
4	63	6.41	6.58	+0.17
5	50*	5.66	5.85	+0.19
6	115	6.79	6.93	+0.14
7	115	6.23	6.45	+0.22
8	115	7.21	7.59	+0.38
9	115	6.89	7.14	+0.25
10	50	6.99	7.00	+0.01
11	55	6.99	7.01	+0.02
12	63	6.91	7.14	+0.23
13	55	6.46	6.47	+0.01
Averages.....		6.42	6.56	+0.14

*Held for six hours.

melted butter sample was adopted. This method provides more complete fat separation than is possible by means of centrifuging. However, in some cases, particularly in the presence of gassy curd, some particles of the curd refuse to settle into the curd-serum mixture and may be lost to the mixed sample of curd-serum. These facts emphasize the importance of definite knowledge regarding the part which the curd plays in the pH determination of butter. It was considered advisable, therefore, to conduct further tests to determine the relation between the aqueous part

of the curd-serum and the curdy portion. These results are shown in tables 6 and 7.

The figures shown in table 6 refer to the aqueous and curdy portions of buttermilk which were separated as completely as possible by repeated centrifuging. The results average 0.14 pH higher for the curdy portion. However, the individual differences between the two portions vary from 0.38 higher for the curdy

TABLE 7

pH of the curd-serum mixture of butter, its aqueous portion, and its curdy portion

SAMPLE NUMBER	CURD-SERUM MIXTURE	AQUEOUS PORTION	CURDY PORTION	DIFFERENCE—EXCESS OF pH OF CURDY PORTION OVER AQUEOUS PORTION
	pH	*pH*	*pH*	*pH*
1	6.20	6.06	6.27	0.21
2	7.25	6.90	7.22	0.32
3	7.31	7.11	7.37	0.26
4	6.07	5.88	5.96	0.12
5	6.39	6.25	6.41	0.16
6	7.25	7.19	7.24	0.06
7		6.14	6.30	0.16
8		5.88	5.96	0.12
9		6.14	6.30	0.16
10		6.03	6.15	0.12
11		6.07	6.09	0.02
Averages for samples 1 to 6...	6.74	6.56	6.74	0.18
Averages for all samples.....	—	6.33	6.48	0.15

portion to 0.04 pH higher for the aqueous portion. These variations are very probably attributable to difference in the relative completeness of separation of the curd from the serum. In order to secure any separation it was necessary to heat the buttermilk. This was done at temperatures varying from 50° to 115°C. for one-half to two and one-half hours. At the higher temperatures the separation was most nearly complete so that in these cases the difference in pH between the aqueous and the curdy portions should be greatest, provided that the curd in-

fluences the pH. This was in fact the case wherever satisfactory separation was possible. These results indicate that the curdy portion does yield a slightly higher pH than the aqueous portion of the buttermilk.

The method of separation of the aqueous and curdy portions from butter was as follows: One pound of butter was melted in the usual way but without shaking at end of heating. With a pipette 25 cc. of a fairly clear aqueous serum could be removed. This represented the aqueous portion. More of the aqueous material was then pipetted out and rejected until curdy material began to enter the pipette. At this point 25 cc. of the thick curdy layer was removed. This represented the curdy portion.

The results on the pH of the aqueous and the curdy portions of butter, as given in table 7, further demonstrate that absence of the curd tends to lower the pH reading. In the eleven churnings studied the differences averaged 0.15 pH and in each individual comparison it was the curdy portion that yielded the higher pH reading. In those comparisons in which the pH of the curd-serum mixture also was determined, the results show close agreement between the whole curd-serum mixture and the curdy portion, while the aqueous portion yielded a consistently lower pH. It is obvious from the above description that, while the aqueous portion used was practically free from curdy material, the curdy portion used contained considerable liquid along with the curd. Had a more complete separation of the curd portion been possible, the difference in pH between the curd portion and the aqueous portion might have been greater.

In comparing the hydrogen ion concentration or true acidities with titratable acidities of butter serums it was observed that in the case of the titratable acidity the serum acid was lower than the curd acid while in the case of true acidity the opposite was the case. Similar findings have been recorded by Whittier and Benton (13) in the case of milk. To further substantiate the results secured by the quinhydrone electrode some of the samples of curdy and serum material were run both by the quinhydrone and hydrogen electrode methods. Exactly the same difference in pH between the curdy portion and the serum portion was

shown by the hydrogen electrode method as by the quinhydrone procedure.

These results, both with buttermilk and with butter, leave little doubt that the curd does have a slight but unmistakable effect on the pH reading. They show that the curd is an integral part of the curd-serum mixture and that this mixture gives a true reading only if all the curd is included in it. This emphasizes the advisability of using as large a sample of butter as possible (about 1 pound) so as to obtain a reasonably large amount of curd-serum mixture.

TABLE 8

pH of whole milk before and after dilution

SAMPLE NUMBER	ACIDITY OF WHOLE MILK	ORIGINAL WHOLE MILK	RATE OF DILUTION		
			1 to 5	1 to 10	1 to 20
	per cent	*pH*	*pH*	*pH*	*pH*
1	0.16	6.58		6.92	7.02
2	0.20	6.45		6.67	6.91
3	0.16	6.63		7.00	7.14
4	0.20	6.25		6.85	6.99
5	0.16	6.61		7.00	7.07
6	0.17	6.58		7.00	7.08
7	0.17	6.50		6.91	7.00
8	0.16	6.60		7.00	7.08
9	0.18	6.59		7.01	7.09
10*	0.16	6.83	7.08	7.18	
Averages.....	0.173	6.532		6.93	7.04
Increase by dilution.....			+0.25	+0.40	+0.51

* No. 10 is not included in the averages but is given to show the relation of the 1 to 5 dilution.

Effect of dilution of sample on pH value. It would be a distinct advantage to be able to dilute the butter sample, so as to reduce the amount of butter required and to make possible the washing of the fat for more complete reclaiming of the curd and acid. If butter serum were well buffered it should be possible to dilute it with several volumes of water without affecting its pH value at all. Such was found to be the case with peptone water and also

with nutrient bouillon as shown by Sharp and McInerney (5). These liquids were diluted as much as 20 times without changing their pH value. However, in the case of milk the above investigators and also Taylor (14) show that the pH value rises with the dilution of the milk. Furthermore, Sharp and McInerney consider that within certain pH ranges the rise was uniform, and upon the strength of their findings they developed certain dilution

TABLE 9

pH of curd-serum mixture from butter undiluted and diluted

CHURNING NUMBER	UNDILUTED CURD-SERUM MIXTURE	DILUTION 1 to 1		DILUTION 1 TO 5		DILUTION 1 to 10		DILUTION 1 TO 50	
		pH value	Increase	pH value	Increase	pH value	Increase	pH value	Increase
	pH		*pH*		*pH*		*pH*		*pH*
711	6.18			6.68	0.50	6.71	0.53	6.85	0.67
1292	6.13					6.73	0.60		
1624	5.68					6.22	0.54		
2078	6.01					6.56	0.55		
2243	6.29					6.75	0.46		
556	4.71			4.99	0.28	5.27	0.56		
1	6.25					6.63	0.38		
2	6.07					6.66	0.59		
1478	5.98	6.28	0.30						
1472	5.12	5.21	0.09						
1049	6.30					6.90	0.60		
1810	5.62					5.75	0.13		
1042	6.22					6.56	0.34		
1032	6.41					6.73	0.32		
2312	5.78					6.29	0.51		
3170	6.09					6.43	0.34		
Average pH increase by dilution.....			0.195		0.39		0.46		0.67

factors for use in colorimetric determinations of the pH on milk. The correction factors adopted ranged from 0.15 pH for milk having a pH around 4.4, to 0.54 pH for milk having a pH range of 6.2 and above. The above data suggested similar experimentation with butter. This study was preceded by dilution experiments on milk for confirming this effect on the pH electrometrically. Table 8 summarizes these results.

The results given in table 8 agree in general with those of the literature cited. Dilution of the milk increased its pH value. The increase progressed in the direction of the dilutions. The pH rose 0.25, 0.40, and 0.51, respectively, for dilutions of 1:5, 1:10, and 1:20.

In the case of butter, there are two possibilities for dilution, namely, dilution of the whole butter and dilution of the curd-

TABLE 10

pH of undiluted curd-serum mixture and of diluted butter

CHURNING NUMBER	UNDILUTED CURD-SERUM MIXTURE	BUTTER DILUTION 1 TO 4		BUTTER DILUTION 1 TO 5		BUTTER DILUTION 1 TO 10	
		pH value	Increase	pH value	Increase	pH value	Increase
	pH		*pH*		*pH*		*pH*
XFM	5.37	5.65	0.28				
1023	7.10	7.60	0.50				
1690	6.33	6.86	0.53				
1678	6.45	6.85	0.40				
956	6.18	6.73	0.55				
1017	6.07	6.49	0.42				
1011	7.16	7.30	0.14				
1446	6.22			6.68	0.46		
1292	6.13			6.58	0.45		
1297	5.94			6.30	0.36		
575	6.32			6.53	0.21	6.86	0.54
1038	6.00					6.56	0.56
2288	5.93					6.57	0.64
1624	5.68					6.27	0.59
2078	6.01					6.66	0.65
2	6.07					6.61	0.54
Average pH increase by dilution.....			0.40		0.37		0.58

serum of the butter after separation and removal from the melted butter. pH values were obtained on dilutions of both the curd-serum mixture and the whole butter, as indicated in tables 9 and 10.

Tables 9 and 10 show that, similarly as in the case of milk, dilution increases the pH value of butter, both when the curd-serum mixture of butter was diluted and when the whole butter

itself was diluted. The increase in pH was greatest for the highest dilutions, but it lacked in uniformity and it was not directly proportional to the rate of dilution.

On the basis of the present limited data, it appears evident that the increase in pH on the diluted sample is not sufficiently consistent for accurate pH determinations. Further work may, however, develop that a certain dilution of the butter may justify the use of a factor for the pH of butter, in a similar way as Sharp and McInerney have suggested for milk for colorimetric deter-

TABLE 11

pH values of butter from different parts of the same churning

NUMBER OF PORTIONS FROM CHURNINGS	CHURNING NUMBERS										
	1422	946	1201	321	1124	1446	180	1297	1292	711	L.B.
1	6.14	7.25	6.35	6.38	6.69	6.22	7.32	5.97	6.13	6.18	6.07
2	6.14	7.26	6.25	6.41	6.60	6.20	7.31	5.94	6.07	6.20	5.96
3	5.98	7.22	6.37	6.38	6.70	6.22	7.24				
4	5.99	7.22	6.35	6.39	6.73						
5	6.09	7.23									
6	6.09										
7	6.07										
8	6.03										
Average pH values	6.07	7.24	6.33	6.39	6.68	6.21	7.26	5.96	6.10	6.19	6.02
Maximum deviation from average	0.09	0.02	0.08	0.02	0.08	0.01	0.06	0.02	0.03	0.01	0.06
Maximum differences between portions	0.16	0.04	0.12	0.03	0.13	0.02	0.08	0.03	0.06	0.02	0.11

minations. Our results show that without such a factor the dilution of butter yields erroneous pH readings.

Uniformity of pH in different portions of the same churning. In obtaining samples of butter for pH determinations the representativeness of the sample taken becomes an item of consideration. This is suggested by the fact that butter is not a product of perfectly uniform composition, hence variations may occur in the hydrogen ion concentration. If such were the case the pH determinations on a single sample of butter would be subject to justifiable criticism. Check tests were, therefore, made on two or more portions of the same churning as shown in table 11.

The figures given in table 11 demonstrate that different portions of butter from the same churnings in most cases do show minor variations in pH. These variations, however, are not sufficiently great to interfere with the value of the pH determinations made on single packages only.

Other factors that may influence the accuracy of pH determinations of butter. One of the factors meriting consideration is the high concentration of the salt in the serum, the serum of average salted butter made in this country being practically a saturated solution of sodium chloride. It is pertinent to suspect the possibility of a "salt error" here, as is sometimes encountered in colorimetric work. Although our present experiment made no attempt to determine this, we have reason to assume that the salt does not cause any material deviation in pH value. Our assumption is based in part at least on the results shown in table 5 and also on additional but unpublished work on salted and unsalted butter made from the same lots of butter granules. In considering the above we are aware of the work done by Sorensen et al. (15) in which they show that the pH by the quinhydrone method on N/100 HCl solutions is depressed slightly by the addition of sodium chloride. For a salt concentration similar to that of the serum of average American salted butter, this depression would appear to be about 0.1 to 0.15 pH.

In addition it is believed that the protein may have an effect on the results obtained by the quinhydrone electrode. Linderström-Lang (16) shows that in the presence of about 0.7 per cent casein in N/20 HCl the quinhydrone electrode differs by about 0.005 pH from the hydrogen electrode. Our results on the curd-serum mixture, table 2, show close agreement between the two electrodes. Whether or not these two factors, i.e., the salt and the protein errors are of similar magnitude in butter, and whether they function in the same manner and direction, or opposite to each other is uncertain.

Another factor has to do with the effect of the fat. The fat may be considered to influence the pH readings in two ways. First, the removal of so large a portion of an ingredient of a product in such a crude way may be open to question. It might

rob the portion actually examined, i.e., the curd-serum mixture, of a sufficient amount of constituents that influence the test, such as acids, curd, and salts, to interfere with the accuracy of the final results. To what extent, if any, they may be a factor, is a question for further study. In the second place, unless special precautions are taken, small portions of fat escape into the curd-serum portion and might thus interfere with the accuracy of the pH reading. Preliminary experimentation in the development of the method of preparation of the butter sample, however, indicates that such small entrained amounts of fat have no noticeable effect on the pH reading.

SUMMARY AND CONCLUSIONS

1. The work herein reported constitutes a study of the factors involved in the electrometric determination of the hydrogen ion concentration of butter.

2. A simple procedure for the preparation of the butter sample and for the application of the quinhydrone electrode to the hydrogen ion determination is described. Its use as a standard method in similar studies is suggested.

3. It is shown that on account of the non-conductive property of butter fat the curd-serum portion must be used in the pH determination.

4. The quinhydrone electrode was found to give essentially the same results on butter as the standard hydrogen electrode.

5. Dilution of the butter or of the curd-serum mixture caused marked changes in the pH values obtained, leading to erroneous results.

6. Removal of the curd from the curd-serum mixture tended to lower the pH value slightly.

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THE HEMOGLOBIN AND TOTAL PHOSPHORUS IN THE BLOOD OF COWS AND BULLS

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Much recent work especially in Germany has been concerned with various anatomical and blood analytical measurements upon milking cows. The physical measurements have been concerned with the size of various portions of the body. The chemical measurements have dealt with such blood constituents as total solids and hemoglobin. Kronacher and his students have reported many such studies (1). In their earlier papers they found a correlation between the fat of the milk and the total solids of the blood in lactating goats. In their later papers (2) they seem to get no correlations between either the physical measurements or the blood constituents and the productive capacities of cows.

The physical measurements of Kronacher (1) included the height at withers, the depth, width and girth of the breast, the hip width, the width and length of pelvis and the rib angle.

The blood constituents and properties that were considered by Kronacher are hemoglobin, dry substance, blood viscosity, plasma viscosity, serum viscosity, erythrocyte sinking rate and alkaline reserve. In both papers Kronacher has called attention to the high positive correlation between the hemoglobin and the total dry substance of the blood.

Since the blood data of Kronacher were not convincing, we decided to run monthly hemoglobin determinations upon the blood of the animals in the Cornell University herd for a period of one year. One thousand and seventy-three analyses were made by the method of Cohen and Smith (3) upon female animals. These included sixty-nine mature cows and twenty-one other younger animals. The mean of all hemoglobin determinations upon female animals was 10.9 $\pm$ 0.86 grams of hemoglobin per 100 cc. of blood. During the course of the same year seventy

determinations were made upon six mature bulls of four different breeds. The mean value for these animals was 12.8 ± 0.8 grams of hemoglobin per 100 cc. of blood. There is a significant difference between the hemoglobin values of the male and female animals. This is the reverse of the findings of Anderson (4), who found females had the higher hemoglobin values. It accords with long established values upon human blood which show that the normal male has more erythrocytes and more hemoglobin per unit volume of blood than the female. In most animal species the male has more hemoglobin per unit volume of blood than the female. Schulz and Krüger (5) have pointed out some exceptions to this rule, however.

Our cows studied included thirty-eight Holsteins, eight Ayrshires, ten Guernseys, and thirteen Jerseys. The mean for each of these breeds is the same within the experimental error. There is no correlation between breed and the hemoglobin level. The following are the mean values for the cows of various breeds in grams of hemoglobin per 100 cc. of blood:

Holstein	10.6 ±0.47
Ayrshire	10.7 ±0.61
Guernsey	10.4 ±0.44
Jersey	10.5 ±0.32

Correlation factors were worked out to determine any relations that might exist between the hemoglobin of the blood and the following variables: the monthly milk production, the monthly fat production, the prolongation of the lactation period, the gestation period. We found no correlation between the hemoglobin and any of these variables. Since some of our age groups were very limited in number, there may have been an age relation here that we could not measure. Since the hemoglobin is known to bear a close relation to the total solids of the blood, we are probably justified in assuming the same result would have been obtained if we had measured total solids. Both total solids and hemoglobin measurements are crude in comparison to the more delicate chemical analytical methods for blood constituents. Our data do not imply that there is no relation between the

chemical constitution of the blood and the production or physiological state of the animal.

An analysis of our data to detect seasonal trends in the hemoglobin level indicated a slight rise during the summer months. Reports from various laboratories have shown increases in the hemoglobin of the blood of lactating cows during the summer months. A close scrutiny of these reports shows that part of them can be neglected either because very few animals were involved in the study or because questionable analytical methods were employed. Hemoglobin has frequently been determined by the use of such standards as the Newcomber glass slide. If this slide is not carefully calibrated the data are usually questionable. In the past six years the author has had occasion to check three of these slides. Only one of these was found to be reasonably accurate.

Since the rise in hemoglobin we found during the summer months was within the range of questionable significance, we decided to repeat a portion of the work. Nearly two years elapsed between our first experiment,and the repetition. In the meantime the composition of the herd previously studied had been altered and a new herd was also available. These new conditions permitted us to consider several variables. The new herd consisting of thirty-nine Holstein cows, was upon the protein experiments being conducted by E. S. Savage and E. S. Harrison. At the time of the first hemoglobin determinations these cows were in their second lactation periods upon the experimental rations. All cows in this herd were fed corn silage as well as clover and timothy hay. One-third received a grain mixture containing 16 per cent protein, another 20 per cent while the last was fed a 24 per cent mixture. Each group of thirteen cows had been upon the experimental diets for approximately eighteen months at the time of our first analyses. This herd gave us an opportunity of detecting any influence these protein-level differences might have upon the hemoglobin.

This was of special interest since we felt the differences detected by others in the summer and winter and hemoglobin might be a measure of the winter management. With inadequate housing or poor feeding during the winter months one might expect a

tendency toward anemia in the spring. In these experiments with different protein intakes one might look for a reflection in the blood proteins in case the minimum was low enough to create a deficiency. Since this herd was allowed sunshine and fresh air in a lot with practically no pasture it afforded an opportunity to study these variables. At the same time another herd of thirty-five milking cows which was turned out to pasture shortly after the middle of May was available.

Kronacher (2) has found the dry matter of the blood of cows increases during the summer by about ten per cent. Van Gelder (6) found that the hemoglobin and red cells of the Swiss mountain cattle increases in the summer. In our second study we sampled the blood of all animals in both experimental herds at the middle of May just before the period of turning them out to the lot or pasture respectively. A second series of samples was taken the middle of July after a two-months period of pasture or dry-lot exposure. Not only hemoglobin, but also iron and total phosphorus determinations were made upon all blood samples. Iron was included as a check upon our hemoglobin methods. In our data we have expressed iron as such. We believe this is preferable to conversion to hemoglobin by the use of a questionable factor.

Several refinements such as longer pipettes and better colorimetry were introduced to decrease the analytical error in the hemoglobin determination. The method of Wong (7) was used for all iron determinations. Total blood phosphorus was determined by the modification of the Denigé method which we have recently described (8). We have measured the total blood phosphorus rather than merely the inorganic phosphate of the plasma since we felt any real increase of any of the various phosphorus compounds in the blood would be reflected in the total. If the total varied, later fractionation studies could be used to detect the class of phosphorus compounds responsible for the shift. We realized, however, that changes in the distribution might occur without changing the total.

In table 1 we have summarized the data of our second experiment. Although the refinement of our hemoglobin method has not altered our mean for the herd upon pasture, which represents

the remnant of the herd upon which our first experiment was made, the values show a much smaller probable error.

Table 1 shows that in our herd there is no influence of either the summer sunshine or of pasture upon the hemoglobin, the iron or the total phosphorus of the blood of milking cows. After eighteen months upon rations that provide varying protein intakes there is no reflection in the blood constituents we have studied. This indicates that the minimum protein intake was adequate for the maintenance of the blood constituents that have

TABLE 1

Iron, phosphorus and hemoglobin in the blood of lactating cows before and after turning to pasture or dry lot in summer

	PASTURE	DRY LOT	HIGH PROTEIN	LOW PROTEIN	MEDIUM PROTEIN
Number of cows	35	39	13	13	13
Total P in May (mgm. per 100 cc.)	19.5±0.12	18.7±0.16	18.8±0.32	18.4±0.26	19.0±0.24
Total P in July (mgm. per 100 cc.)	19.7±0.16	18.8±0.15	18.5±0.22	18.7±0.29	19.3±0.25
Fe in May (mgm. per 100 cc.)	39.5±0.39	38.7±0.48	39.2±0.89	38.9±0.92	38.9±0.64
Fe in July (mgm. per 100 cc.)	37.6±0.34	39.2±0.49	38.7±0.62	39.4±0.75	39.3±0.12
Hb in May (grams per 100 cc.)	11.0±0.06	11.7±0.14	11.7±0.19	11.7±0.29	11.9±0.19
Hb in July (grams per 100 cc.)	10.8±0.09	11.2±0.14	11.0±0.21	10.9±0.19	11.6±0.18

been measured. We offer no explanation for the contrasts between our results and those of Kronacher other than the possibility of differences in herd management during the winter months. Our data show the very small differences that are found in the mean values for the hemoglobin, iron or total phosphorus values of the blood if an adequate number of animals is used in an experiment.

SUMMARY

By means of over a thousand determinations we have shown that the blood of the normal cow contains 10.9 ± 0.86 grams of

hemoglobin per 100 cc. of blood. The blood of mature bulls is significantly higher in hemoglobin than that of cows. It contains 12.8 ± 0.8 grams per 100 cc. There is no relation between the hemoglobin of the cow and the following variables: breed, milk production, fat production, and the prolongation of the lactation period. There is no change in the hemoglobin of the blood that can be related to the period of gestation or lactation. No differences were found in total phosphorus, iron and hemoglobin in the blood of seventy-four cows at the close of the winter months and after a two-months' period of exposure to pasture or sunshine. No influence of an eighteen-months' feeding period with three levels of protein upon the hemoglobin, iron or total phosphorus of the blood of lactating cows could be found.

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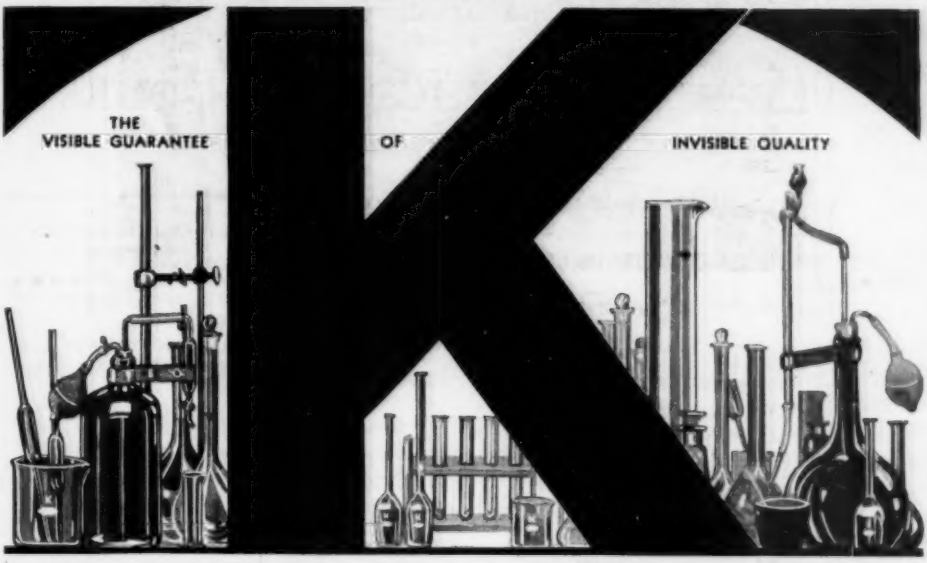

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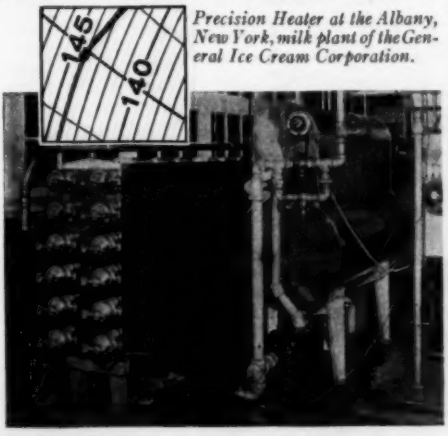

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